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Comparative electrophoretic investigation on *Rana balcanica* and *Rana ridibunda* from northern Greece

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A total of 315 water frogs pertaining to either *Rana ridibunda* or *Rana balcanica* was collected at 28 sites distributed over northern Greece. The intra- and interspecific variability of the gene pools was assessed by applying vertical polyacrylamide gel electrophoresis on samples of blood, liver, skeletal and heart muscle. We compared the allelic variation of eight enzyme loci and of two non-enzymatic proteins among populations and species using the UPGMA-, Fitch-Margoliash- and maximum-likelihood-methods. The variability between conspecific populations was considerably smaller than the interspecific variability. Moreover, at the ADA locus alternatively fixed alleles were found in *R. balcanica* and *R. ridibunda*. Thus, the species status of the bioacoustically detected *R. balcanica* was confirmed.

INTRODUCTION

Until recently *Rana ridibunda* Pallas, 1771 has been considered a monotypic species with a wide geographical range including northern Africa, Europe and the western regions of Asia (MERTENS & WERMUTH, 1960; GÜNTHER, 1990). However, intensive bioacoustic studies on features of the mating calls recorded in populations in Armenia (SCHNEIDER & EGIASARJAN, 1990), Egypt (AKEF & SCHNEIDER, 1989), Greece (SCHNEIDER & SOFIANIDOU, 1985; SCHNEIDER et al., 1984), Israel (NEVO & SCHNEIDER, 1983), Kazakhstan (SCHNEIDER & EGIASARJAN, 1991) and Turkey (JOERMANN et al., 1988) led to the unequivocal distinction of three different species within this geographical range (SCHNEIDER & SINSCH, 1992): *Rana ridibunda* in the terra typica restricta in Kazakhstan, and in Armenia, eastern Greece and Bulgaria, *Rana levantina* (SCHNEIDER et al., 1992) in Egypt, Israel and western

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Turkey, and *Rana balcanica* (SCHNEIDER et al., 1993) in Greece, Albania and Yugoslavia. Additional morphometric comparisons revealed slight morphological differentiations between these species (SCHNEIDER et al., 1992, 1993). Analyses of allozyme variation among the bioacoustically detected taxa corroborated their species status (NEVO & FILIPUCCI, 1988; NEVO & YANG, 1982; SINSCH & EBLENKAMP, 1994; SOFIANIDOU, unpubl. data).

All previous studies on the allozyme variation between the new species *R. balcanica* and *R. ridibunda* have been performed on a rather large geographical scale and focused on resolving the taxonomic problems by comparison of bioacoustically classified populations. In the present investigation, allozyme electrophoresis was used to specify more precisely the distribution of *R. balcanica* and *R. ridibunda* in Greece. Our aims were: (1) to assess the genetic variability of each species on a small geographical scale by collecting samples along an east-west transect through northern Greece; (2) to compare the intraspecific and interspecific genetic variation; (3) to investigate the stability of genotypes at the neighbouring limits of geographical distribution at the Nestos River; and (4) to compare the results with those of the bioacoustic studies.

MATERIAL AND METHODS

At 28 sites distributed over northern Greece (Table I, fig. 1), we collected a total of 315 individuals of *Rana balcanica* and *Rana ridibunda* during three successive breeding periods. At the Epeiros sites where *R. balcanica* occurs syntopically with *R. epeirotica*, species identification was based on the mating calls. Specimens were measured, sexed and numbered. Samples of blood (plasma and red cell hemolysates), liver, skeletal and heart muscle were obtained from each specimen and stored at −35°C until electrophoretic analysis. The carcasses remaining after tissue sampling were preserved and deposited in the collection of the Laboratory of Zoology at the University of Thessaloniki.

For electrophoresis, we employed the vertical polyacrylamide gel technique using the SE 600 Hoefer apparatus. Gels included a stacking and a separating portion and were 1.5 mm or 0.75 mm thick. Electrophoresis was conducted under refrigeration (4-6°C). Mostly, a discontinuous buffer system was used (stacking gel buffer Tris-HCl, pH 6.8; resolving buffer Tris-HCl, pH 8.8; reservoir buffer Tris-glycine, pH 8.3), whereas in case of the LDH we employed a borate pH 8.2 system (PASTEUR et al., 1988). The samples (plasma and aqueous extracts) of tissues were run in gels with two different percentages of monomer in the separating gel (7.5 % T − 10 % T). The power supply was set for constant amperage (30 mA), and the duration of electrophoresis varied from 3-6 h depending on the protein.

Enzymes and other proteins were stained using standard procedures (PASTEUR et al., 1988). Proteins of different individuals were compared side by side on the same gel to avoid errors due to the slightly varying absolute mobilities in different gels. Enzyme systems examined were: adenosine deaminase (ADA, EC 3.5.4.4), α-glycerophosphate dehydrogenase (α-GPD, EC 1.1.1.8), lactate dehydrogenase (LDH, EC 1.1.1.27), malate

Source : MNHN, Paris

Table I. - List of sampling localities in Northern Greece and number of water frogs collected for protein analyses. The spatial relations between the sites are given in fig. 1.

	Locality	Number of individuals per sample		
		a	b	c
	Epeiros			
1.	Panvotis Lake (a and b at the same locality)	20	10	
2.	Louros River	10		
3.	Parakalamos (upper part of the Kalamas River)	6		
4.	Sagiada Wetland	20		
5.	Nea Selefkeia marsh	6		
6.	Kalodiki, swampy lake	10		
	Macedonia			
7.	Axios River, river side pond	20		
8.	Axios River, river bank	6		
9.	Doirani Lake	4		
10.	Gallikos River, estuary region	20		
11.	Gallikos River, 35 km north of site 10	6		
12.	Thessaloniki, brooklet in the forest Seih-Su	4		
13.	Lankadas, small river	10		
14.	Kerkini Lake	6		
15.	Agion Oros, Moni Zographu	3		
16.	Thasos Island	5		
17.	Nestos River, river bank	12		
18.	Nestos River, river side pond	6		
	Thrace			
19.	Kompsatos River	4		
20.	Thermes of Echinos	3		
21.	Vistonis Lake	20		
22.	Komotini, small river	13		
23.	Evros Delta	8		
24.	Samothraki Island	6		
25.	Erythropotamos River (near Didymoteicho)	8		
26.	Kufovuno, small branch of Erythropotamos	8		
27.	Valtos, Orestiada, brook (a and b at the same locality)	16	5	
28.	Ardas River, at Komara (a, b and c at the same locality)	22	7	9
	Total	284	22	9

Source : MNHN, Paris

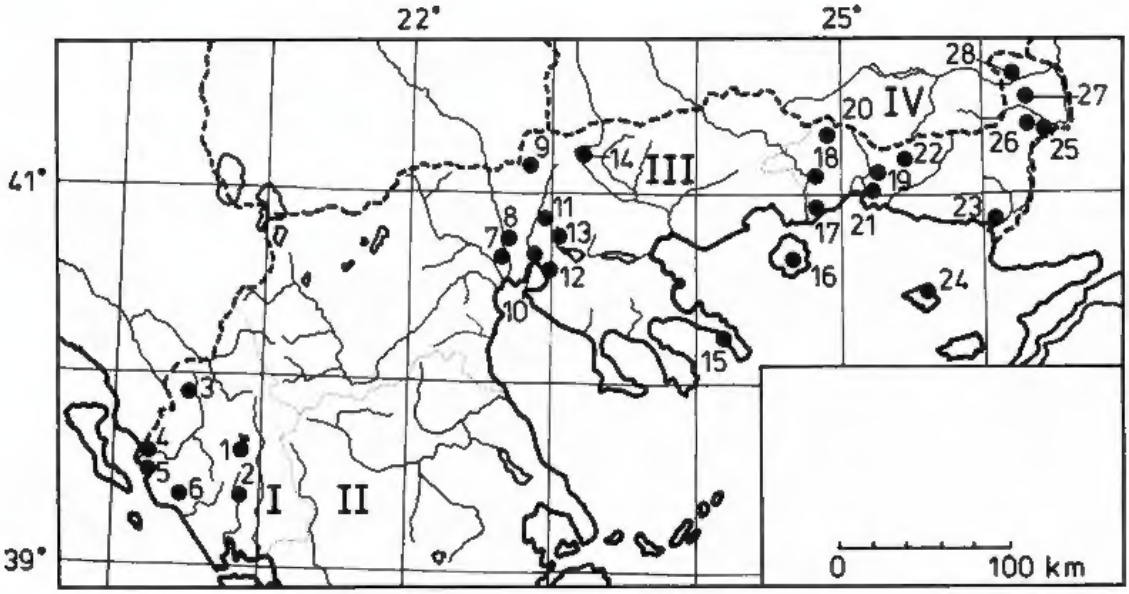

Fig. 1. – Map of northern Greece indicating the spatial relations between the sampling sites (dots and numbers) of water frogs. Table I gives the names of the sampling sites and the number of individuals collected. I: Epeirus; II: Thessalia; III: Macedonia; IV: Thrace.

dehydrogenase (MDH, EC 1.1.1.37), mannose phosphate isomerase (MPI, EC 5.3.1.8), 6-phosphogluconate dehydrogenase (6-PGD, EC 1.1.1. 37), phosphoglucomutase (PGM, EC 2.7.5.1). Additionally, the electromorphs of plasma albumin (ALB) and of a soluble muscle protein (MProt) were analyzed.

Several other loci such as MDH-2 or MProt-2 and non-enzymatic muscle and plasma proteins were scored in preliminary analyses and proved to be monomorphic. Since this study focused on taxonomic distinction between the two species and not on an estimate of their phylogenetic distance, we refrained from including the presumptive monomorphic loci into the main study.

Multiple loci were numbered according to the mobility of their products from anode to cathode. Stainable bands corresponding to the alleles of one presumptive locus were assigned letters according to their mobility, beginning with the band closest to the anode. Average heterozygosity per locus (H_o = observed frequency; H_e = expected frequency), proportion of polymorphic loci (P %), and the mean number of alleles per locus (A) were calculated for each sample. We used the G-test to detect deviations of the observed heterozygosity from the Hardy-Weinberg equilibrium, and the non-parametric Wilcoxon-Mann-Whitney U test (two-tailed) for the interspecific comparisons of H_o and P %. Cavalli-Sforza's chord distance (Cavalli-Sforza & Edwards, 1967) was calculated for all pairwise combinations of samples using the program GENDIST 3.4 of the package PHYLIP, version 3.4 (Felsenstein, 1985). Although we did not consider presumably monomorphic loci, these estimates of genetic distance are useful to resolve the relative

Source : MNHN, Paris

genetic relationships between the examined populations by computing rooted and unrooted trees based on four algorithms: (1) UPGMA method (program NEIGHBOR 3.41); (2) FITCH-MARGOLIASH method assuming equal rates of evolutionary change in all lineages (KITSCH 3.41); (3) FITCH-MARGOLIASH method without evolutionary clock (FITCH 3.41); (4) maximum-likelihood method (CONTML 4.42). All calculations are based on the cited programs of the package PHYLIP (FELSENSTEIN, 1985).

RESULTS

ALLELIC VARIATIONS OF PROTEINS

A total of 10 presumptive loci (enzymes: ADA, α-GPD, LDH-1, LDH-2, MDH-1, MPI, 6-PGD, PGM-2; non-enzymatic proteins: ALB, MProt) were scored in 32 samples of frogs from 28 sites (Table II). Except for one (MDH-1), all loci were polymorphic, producing two to four bands of distinct electrophoretic mobility which we consider the results of the activity of different alleles of the same locus. The corresponding frequencies of the alleles are listed in Table II. Observed heterozygosity did not deviate significantly from expectations (H_e) in all but two populations (Vistonis Lake and Komotini River, significant deficit of heterozygotes). However, the average observed heterozygosity H_o ($P = 0.00388$, Wilcoxon U test), the proportion of polymorphic loci P % ($P = 0.000256$, U test), and the mean number of alleles per locus A ($P = 0.01723$, U test) were significantly greater in *R. ridibunda* populations than in *R. balcanica* populations.

The following account of the polymorphic loci demonstrates that (1) alternatively fixed alleles at the ADA locus permitted an unequivocal distinction between *R. balcanica* and *R. ridibunda* populations, and (2) there was a clear geographical variation of allele frequencies at other loci, which distinguished the *R. balcanica* populations of Epeiros from their conspecifics in Macedonia. We refrained from speculations on presumptive homologies of electromorphs detected in the present study with those in studies of other authors because different electrophoretic conditions render such comparisons unreliable.

Allozymes

Adenosine deaminase

The two alleles permitted an unequivocal electrophoretic discrimination between samples of *R. balcanica* from Epeiros and Macedonia and *R. ridibunda* from Thrace. *Rana balcanica* populations were fixed for the a allele, *R. ridibunda* populations for the b allele.

α-glycerophosphate dehydrogenase

All samples collected from *R. balcanica* populations were monomorphic for the a allele. The only exception was one of the two samples from the Nestos River, in which we also detected the b allele at a frequency of 25 %. In contrast, the b allele predominated in the *R. ridibunda* populations.

Source : MNHN, Paris

Table II. - Allele frequencies at ten presumptive loci in samples of water frogs from 25 localities through northern Greece. Numbers refer to the sites listed in Table I. Replicate samples are identified by, e.g., 1a and 1b for site 1. P %: relative frequency of polymorphic loci; A: average number of alleles per locus; H_e: relative frequency of expected heterozygosity; H_o: relative frequency of observed heterozygosity.

		1a	1b	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ADA	a	1.00	1.00	1.00	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-GPD	a	1.00	1.00	1.00	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LDH-1	a	1.00	1.00	1.00	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67
	b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.33
LDH-2	a	1.00	1.00	1.00	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	-	1.00	1.00	1.00	1.00
	b	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-
MDH-1		1.00	1.00	1.00	1.00	1.00	1.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
MPI	a	0.70	0.70	0.80	0.83	0.70	0.7	0.65	0.95	1.00	1.00	0.90	0.92	1.00	0.95	0.92	1.00
	b	0.30	0.30	0.20	0.17	0.30	0.3	0.35	0.05	-	-	0.10	0.08	-	0.05	0.08	-
6-PGD	a	-	-	-	-	-	-	-	0.05	0.08	-	0.10	-	-	0.05	0.08	-
	b	1.00	1.00	1.00	1.00	1.00	1.0	1.00	0.90	0.92	1.00	0.85	1.00	1.00	0.95	0.92	1.00
	c	-	-	-	-	-	-	-	0.05	-	-	0.05	-	-	-	-	-
PGM-2	a	0.35	0.40	0.30	0.33	0.30	0.17	0.05	0.10	-	-	0.05	-	-	0.05	0.08	-
	b	0.65	0.60	0.70	0.67	0.70	0.83	0.95	0.90	1.00	1.00	0.95	1.00	1.00	0.95	0.92	1.00
ALB	a	-	-	-	-	0.10	-	0.10	0.03	-	-	-	-	-	0.08	-	
	b	0.85	0.90	0.70	0.67	0.60	0.67	0.60	0.47	0.50	0.50	0.40	0.40	0.50	0.40	0.42	0.50
	c	0.15	0.10	0.30	0.33	0.30	0.33	0.30	0.50	0.50	0.50	0.43	0.60	0.50	0.60	0.50	0.50
	d	-	-	-	-	-	-	-	-	-	0.17	-	-	-	-	-	
MProt	a	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-
	b	1.00	1.00	1.00	1.00	1.00	1.0	1.00	0.60	0.67	0.62	0.63	0.65	0.50	0.60	0.50	0.50
	c	-	-	-	-	-	-	-	0.37	0.33	0.38	0.37	0.35	0.50	0.40	0.50	0.50
P %		0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.5	0.3	0.2	0.5	0.3	0.2	0.5	0.5	0.3
A		1.3	1.3	1.3	1.3	1.4	1.3	1.4	1.8	1.3	1.2	1.7	1.3	1.2	1.5	1.6	1.3
H_e		0.11	0.11	0.12	0.12	0.14	0.11	0.11	0.15	0.10	0.10	0.16	0.11	0.10	0.12	0.15	0.14
H_o		0.11	0.10	0.11	0.10	0.13	0.08	0.10	0.11	0.10	0.10	0.16	0.10	0.10	0.11	0.11	0.15

Source : MNHN, Paris

Table II. - Continuation.

		16	17	18	19	20	21	22	23	24	25	26	27a	27b	28a	28b	28c
ADA	a	1.00	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-		
	b	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
α-GPD	a	1.00	0.75	1.00	0.25	0.33	0.40	0.29	0.38	0.50	0.25	0.30	0.30	0.20	0.27	0.34	0.50
	b	-	0.25	-	0.75	0.67	0.60	0.71	0.62	0.50	0.75	0.70	0.70	0.80	0.73	0.66	0.50
LDH-1	a	1.00	0.83	0.67	-	-	0.45	0.31	0.13	0.50	-	-	0.10	-	0.27	0.43	0.44
	b	-	-	0.33	0.25	1.00	-	-	-	-	-	-	-	-	-	-	0.17
	c	-	0.17	-	0.75	-	0.55	0.69	0.87	0.50	1.00	1.00	0.90	1.00	0.73	0.57	0.39
LDH-2	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MDH-1		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
MPI	a	0.90	0.60	0.50	0.50	0.33	0.25	0.31	0.25	0.50	0.25	0.30	0.30		0.27		
	b	0.10	0.40	0.50	0.50	0.67	0.75	0.69	0.75	0.50	0.75	0.70	0.70		0.73		
6-PGD	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	b	1.00	0.83	0.87	-	-	-	-	-	-	-	-	-	-	0.14	0.33	-
	c	-	0.17	0.13	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.86	0.67	1.00
PGM-2	a	-	0.08	-	0.62	0.67	0.52	0.69	0.69	0.67	0.75	0.75	0.70	0.80	0.75		
	b	1.00	0.92	1.00	0.38	0.33	0.48	0.31	0.31	0.33	0.25	0.25	0.30	0.20	0.25		
ALB	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	b	0.40	0.43	0.50	0.38	0.50	0.35	0.37	0.38	0.33	0.50	0.30	0.34	0.30	0.39	-	0.30
	c	0.60	0.54	0.50	0.62	0.50	0.65	0.63	0.62	0.67	0.50	0.70	0.63	0.70	0.61	0.67	0.40
	d	-	0.03	-	-	-	-	-	-	-	-	-	0.03	-	-	0.33	0.20
MProt	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	b	0.60	0.25	0.13	-	-	0.10	-	-	0.17	-	-	-	-	0.05	-	
	c	0.40	0.75	0.87	1.00	1.00	0.90	1.00	1.00	0.83	1.00	1.00	1.00	1.00	0.95	1.00	
P %		0.3	0.7	0.5	0.5	0.4	0.6	0.5	0.5	0.6	0.5	0.4	0.5		0.7		
A		1.3	1.8	1.5	1.5	1.4	1.6	1.5	1.5	1.6	1.4	1.4	1.6		1.7		
H_e		0.11	0.22	0.19	0.22	0.18	0.27	0.22	0.20	0.21	0.16	0.16	0.20		0.26		
H_o		0.10	0.22	0.17	0.15	0.20	0.19	0.10	0.13	0.20	0.13	0.15	0.17		0.20		

Lactate dehydrogenase

Three alleles were detected at the LDH-1 locus. However, in 16 of the 18 *R. balcanica* populations this locus was monomorphic for the a allele. Only at Agion Oros did the c allele, and at the Nestos River the b and c alleles, also occur. In contrast, the c allele dominated over the a and b alleles in all *R. ridibunda* populations, except of (1) Samothraki where all 6 individuals were heterozygous (a/c) and of (2) Komara (replicate sample 28c) where the a allele dominated. Heterozygotes of the a/c constitution (6 at Samothraki, 2 at Nestos River, 2 at Lake Vistonis, 1 at Valtos, 5 at Komara) showed the

Source : MNHN, Paris

typical five-banded pattern, whereas, due to the small difference in electrophoretic mobility of a and b gene products, a/b heterozygotes (1 at Nestos River, 1 at Komara) showed only two readily distinguishable bands.

The LDH-2 locus was monomorphic in all but one populations. The extremely rare b allele was detected during a repeated sampling in six individuals from the Gallikos River.

Mannose phosphate isomerase

There were conspicuous geographical differences in the frequencies of the two detected alleles at this locus. The *R. balcanica* populations of Macedonia were almost monomorphic for the a allele, whereas the frequency of the b allele increased to about 30 % in the conspecific populations of Epeiros. In the *R. ridibunda* populations, the frequency of the b allele ranged between 50 and 76 %. Notable exceptions to this general pattern were the *R. balcanica* samples from the Nestos River, with allele frequencies resembling those of *R. ridibunda* populations.

6-phosphogluconate dehydrogenase

The three alleles detected at the 6-PGD locus again showed a clear geographical segregation. The *R. balcanica* populations of Epeiros were monomorphic for the b allele, whereas low frequencies of the a and c alleles were present in the conspecific populations of Macedonia besides the dominant b allele. In contrast, the *R. ridibunda* populations of Thrace were monomorphic for the c allele (except for Komara: low frequencies of the b allele).

Phosphoglucomutase

As in the allelic distribution in the MPI, the frequencies of the two alleles at the PGM-2 locus varied geographically. The *R. balcanica* populations of Macedonia were almost monomorphic for the b allele, whereas the frequency of the a allele increased to about 30 % in the conspecific populations of Epeiros. In the *R. ridibunda* populations, the frequency of the a allele increased even more, to about 70 %. The a/b heterozygotes showed a broad band ranging from the positions of the homozygote a and b bands.

Non-enzymatic proteins

Albumin

The frequencies of the most common alleles, b and c, varied clinally along an east-west axis in *R. balcanica* and in *R. ridibunda*. Allele d was frequent only at Komara and at Gallikos River, whereas it was rare at Nestos River and at Valtos and absent in all other samples. Allele a occurred in low frequencies at some localities in Epeiros and Macedonia, i.e. exclusively in populations of *R. balcanica*.

Muscle protein

The three different stainable bands found in the fraction of soluble muscle protein showed geographically and interspecifically varying frequencies. In the *R. balcanica*

Source : MNHN, Paris

populations of Epeiros, only the b band was detected, whereas in the populations of Macedonia the c band reached frequencies ranging from 33 to 87 %. The rare a band was found only once, at the Axios River. Finally, the c band was often the only one detected in the *R. ridibunda* populations, but at some sites low frequencies of the b bands were also found. Frequent two-banded heterozygotes of the b/c type indicated that the three bands are probably due to allelic variation of the same locus.

GENETIC DIFFERENTIATION

The genetic variation between samples collected at the same or nearby sites (< 1 km), and between samples from different localities, was assessed by calculating CAVALLI-SFORZA's chord distance (Table III). However, we excluded the replicate samples from Valtos and Komara (sites 27 and 28 in Thrace) because of missing data which might bias the comparison

Random effects of sampling

The chord distances between samples collected at the same site (Panvotis Lake), and between those collected in close vicinity (Axios River, Nestos River), ranged between 0.001 and 0.133. These three sites were inhabited by *R. balcanica* populations.

Intraspecific variation

The chord distances between the 18 *R. balcanica* populations ranged from 0.005 between the Louros and Kalamas Rivers to 0.462 between the geographically distant localities Panvotis Lake and Gallikos River. Within the major geographical regions Epeiros and Macedonia, genetic variation was considerably smaller. The six populations from Epeiros were genetically very similar, with chord distances between 0.005 and 0.056 (median: 0.018). The twelve populations from Macedonia were less uniform, with chord distances ranging between 0.012 and 0.438 (median: 0.119). Finally, the intraspecific genetic variation among the ten *R. ridibunda* populations from Thrace varied with chord distances of 0.006-0.324 (median: 0.072) within the range of the *R. balcanica* populations from Macedonia.

Interspecific variation

Finally, the comparison between the populations of *R. balcanica* and *R. ridibunda* yielded chord distances in the range of 0.553 to 1.625 (median: 1.197). Even the smallest chord distance between any interspecific pair was still greater than the greatest distance between pairs of conspecific populations

Grouping of populations

All populations examined were grouped with respect to their genetic similarity using four algorithms which are commonly applied in the reconstruction of phylogenetic relationships. All algorithms led to identical or similar groupings of populations.

Source MNHN, Paris

Table III - CAVALLI-SFORZA's chord distances between the water frog populations of 25 sampling sites. The replicate samples 27b, 28b and 28c were excluded because not all loci were scored

	1a	1b	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27a	28a
1a	-	0.001	0.007	0.010	0.022	0.013	0.045	0.137	0.187	0.184	0.161	0.442	0.207	0.146	0.161	0.259	0.163	0.282	0.336	1.339	1.321	1.041	1.193	1.233	0.972	1.362	1.369	1.268	1.048
1b		-	0.013	0.016	0.028	0.022	0.056	0.151	0.205	0.202	0.175	0.462	0.225	0.163	0.176	0.277	0.183	0.298	0.354	1.346	1.326	1.051	1.200	1.240	0.980	1.365	1.376	1.275	1.053
2			-	0.005	0.017	0.006	0.037	0.108	0.146	0.144	0.134	0.406	0.167	0.111	0.132	0.218	0.127	0.265	0.321	1.331	1.325	1.039	1.192	1.235	0.962	1.370	1.365	1.267	1.050
3				-	0.018	0.008	0.042	0.106	0.145	0.142	0.134	0.406	0.165	0.109	0.131	0.217	0.128	0.268	0.328	1.329	1.326	1.040	1.191	1.235	0.959	1.370	1.364	1.266	1.049
4					-	0.018	0.018	0.114	0.175	0.173	0.152	0.424	0.196	0.132	0.126	0.247	0.145	0.271	0.325	1.333	1.327	1.032	1.188	1.229	0.964	1.366	1.360	1.262	1.045
5						-	0.021	0.108	0.140	0.137	0.127	0.389	0.160	0.106	0.128	0.212	0.110	0.248	0.290	1.337	1.329	1.032	1.194	1.235	0.970	1.374	1.370	1.269	1.054
6							-	0.112	0.145	0.142	0.138	0.390	0.165	0.120	0.119	0.217	0.110	0.259	0.283	1.379	1.372	1.065	1.237	1.277	1.014	1.420	1.415	1.312	1.099
7								-	0.039	0.045	0.038	0.325	0.047	0.018	0.017	0.099	0.040	0.139	0.168	1.145	1.161	0.888	1.022	1.069	0.819	1.216	1.196	1.097	0.916
8									-	0.012	0.055	0.311	0.016	0.017	0.040	0.068	0.029	0.207	0.214	1.317	1.344	1.061	1.207	1.257	0.988	1.407	1.386	1.284	1.099
9										-	0.070	0.299	0.021	0.023	0.049	0.054	0.016	0.191	0.195	1.305	1.332	1.052	1.195	1.245	0.981	1.395	1.374	1.272	1.085
10											-	0.342	0.072	0.037	0.047	0.124	0.056	0.133	0.174	1.169	1.182	0.902	1.042	1.088	0.841	1.237	1.217	1.097	0.939
11												-	0.302	0.301	0.325	0.354	0.286	0.438	0.438	1.547	1.566	1.276	1.425	1.472	1.218	1.625	1.601	1.500	1.311
12													-	0.025	0.047	0.052	0.018	0.180	0.180	1.279	1.306	1.035	1.169	1.219	0.967	1.369	1.348	1.246	1.065
13														-	0.015	0.076	0.016	0.149	0.168	1.212	1.232	0.953	1.090	1.138	0.887	1.289	1.264	1.165	0.979
14															-	0.099	0.037	0.148	0.165	1.184	1.199	0.930	1.058	1.105	0.868	1.253	1.232	1.133	0.952
15																-	0.069	0.159	0.222	1.137	1.306	0.948	1.062	1.085	0.887	1.205	1.184	1.106	0.951
16																	-	0.143	0.140	1.245	1.263	0.975	1.121	1.167	0.921	1.321	1.298	1.197	1.010
17																		-	0.133	0.773	0.882	0.564	0.650	0.684	0.553	0.800	0.790	0.697	0.562
18																			-	0.946	0.857	0.803	0.910	0.937	0.799	1.069	1.058	0.971	0.833
19																				-	0.151	0.132	0.087	0.068	0.147	0.053	0.049	0.063	0.113
20																					-	0.309	0.289	0.289	0.324	0.290	0.293	0.294	0.318
21																						-	0.025	0.038	0.017	0.104	0.099	0.050	0.039
22																							-	0.009	0.043	0.052	0.050	0.015	0.029
23																								-	0.062	0.025	0.022	0.006	0.035
24																									-	0.134	0.122	0.072	0.053
25																										-	0.007	0.024	0.072
26																											-	0.020	0.071
27a																												-	0.041
28a																													-

Source : MNHN, Paris

Therefore, we present the unrooted FITCH-MARGOLIASH-tree (fig. 2) as an example of the three algorithms based on CAVALLI-SFORZA's chord distance matrix and the unrooted maximum-likelihood-tree (fig. 3) directly calculated from the allele frequencies. The best FITCH-MARGOLIASH dendrogram with contemporary tips out of 4394 examined had a sum of squares of 50.023 with a corresponding average percent standard deviation of 24 851 The best unrooted FITCH-MARGOLIASH tree out of 7093 examined showed a better fit (sum of squares: 14 611; average percent standard deviation: 13.431). The best unrooted maximum-likelihood tree out of 1931 examined had a ln likelihood of 760.407.

Within the *R. balcanica* populations, there were two major clusters of populations corresponding to the samples from Epeiros (sites 1-6) and to those from Macedonia (7-16). Despite the occurrence of the unique LDH-2 allele b in a sample from the Gallikos River (site 11), this sample was included in the Macedonia cluster in the two unrooted trees (figs. 2-3), whereas the two rooted dendrograms (UPGMA, FITCH-MARGOLIASH with contemporary tips) failed to recognize this association and placed this population as an outgroup of all other *R. balcanica* populations. All reconstructions coincided in assigning the population from the Nestos River a position apart from the main clusters, but clearly within the branch of *R. balcanica*. Nevertheless, Balkan frogs from the Nestos River differed from those of all other sites by the introgression of *R. ridibunda* genome (e.g. α-GPD, LDH-1, MProt, 6-PGD).

Eight out of ten *R. ridibunda* populations (sites 21-28) joined one major cluster similar to those representing *R. balcanica* in Epeiros and Macedonia. However, again two populations (sites 19-20: Kompsatos River and Thermes of Echinos) differed considerably from all other conspecifics due to the presence of a rare allele of the LDH-1.

DISCUSSION

The allelic variation of proteins among 28 populations of water frogs in northern Greece corroborates the previous bioacoustic finding that *R. balcanica* inhabits the region west of the Nestos River and *R. ridibunda* the eastern region (SCHNEIDER & SINSCH, 1992). The populations at sites 1-18 (Table I) correspond to *R. balcanica*, those at sites 19-28 to *R. ridibunda*. The alternative fixation of the ADA alleles permitted an unequivocal biochemical distinction of the two species under these electrophoretic conditions

As we deliberately selected the polymorphic loci for our purpose of taxonomic distinction, and did not follow the presumably monomorphic loci detected, we chose CAVALLI-SFORZA's chord distance instead of NEI's (1972) genetic distance as a measure of genetic differentiation. The calculation of NEI's genetic distances from our data set would have led to an overestimation of the real genetic differentiation due to restriction on polymorphic loci. Therefore, our study presents relative values of genetic differentiation among the populations studied which cannot be compared directly with NEI's distances published elsewhere. However, CAVALLI-SFORZA's chord distance is a useful measure to decide whether the genetic differentiation between *R. balcanica* and *R ridibunda* supports the proposed species status or not.

Source MNHN, Paris

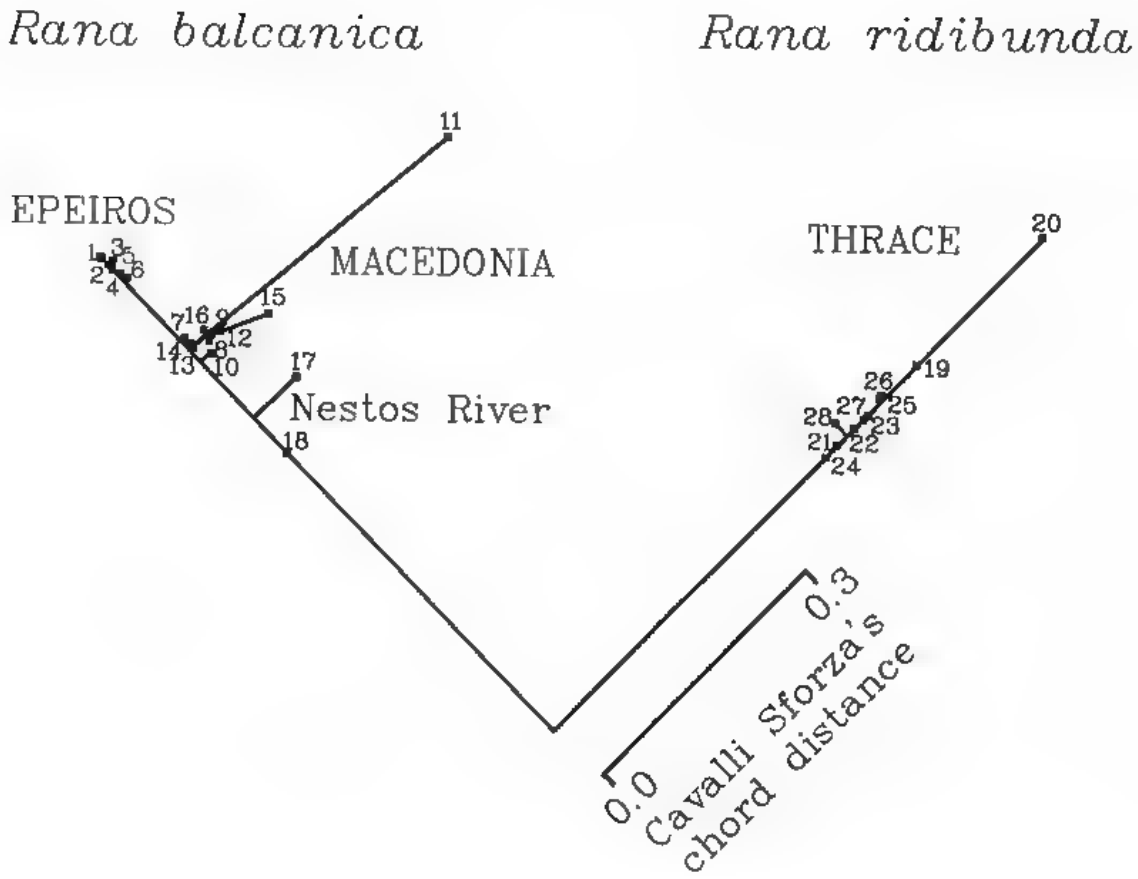

Fig. 2. – FITCH-MARGOLIASH tree (best one out of 7093 examined, sum of squares: 14.61, average percent standard deviation: 13.43) based on CAVALLI-SFORZA's chord distance matrix (Table III) of all pairwise combinations of the samples. Due to the absence of an outgroup this tree is arbitrarily rooted at the mean distance between the most similar populations of *R. balcanica* and *R. ridibunda*. The localities corresponding to the numbers of the populations are listed in Table I

INTRASPECIFIC GENETIC DIFFERENTIATION

The chord distances separating conspecific populations within a given region (Epeiros, Macedonia, Thrace) are low, but increase with geographical distance as should be expected. HOTZ & UZZELL (1982) also found low NEI's distances (D = 0.00-0.02) in water frogs of southwestern Greece (now classified as *R. balcanica*), and NEVO & YANG (1982) in water frogs of Israel (now classified as *R. levantina*; D = 0.006-0.056). This genetic homogeneity is probably due to the extensive exchange of individuals among neighbouring populations. This is not surprising as *R. balcanica* is known to migrate over large distances (SOFIANIDOU & SCHNEIDER, 1989), as do other European water frogs (15 km within one activity period in adult *R. lessonae* and *R. esculenta*; TUNNER, 1992), and juveniles usually disperse over even larger distances than adults (SINSCH, 1991, 1992).

Source MNHN, Paris

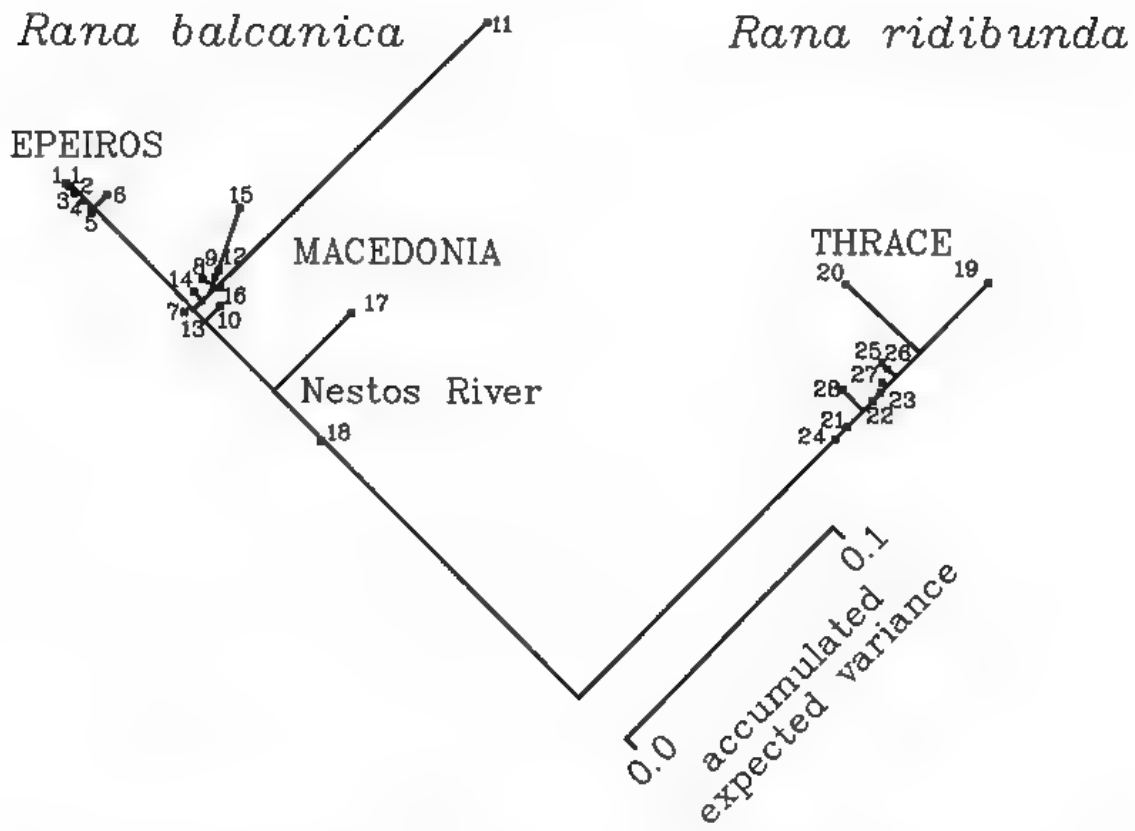

Fig. 3. – Maximum-likelihood tree (best one out of 1931 examined with ln likelihood: 760.407) based on the allele frequencies (Table II) of each sample. Due to the absence of an outgroup this tree is arbitrarily rooted at the mean distance between the most similar populations of *R. balcanica* and *R. ridibunda* The localities corresponding to the numbers of the populations are listed in Table I.

Nevertheless, we detected two notable exceptions to the general pattern, the *R. ridibunda* populations at the Kompsatos River (site 19) and Thermes of Echinos (site 20), and the *R balcanica* populations at the Nestos River (site 17-18) These four populations conspicuously deviated from the other conspecific ones, but still were clearly assignable to their respective species because of the alternatively fixed alleles of the ADA-locus. The *R. ridibunda* from the Kompsatos River and Thermes of Echinos differed from all others in the frequency of the b allele at the LDH-1 locus. Both localities are situated in the highlands, and altitude associated with warm water at the Thermes of Echinos may represent a selective pressure in favour of this rare allele.

At the Nestos River, the situation is more complicated. We know from bioacoustic and allozyme studies (SCHNEIDER et al., 1993, SINSCH & EBLENKAMP, 1994) that *R. balcanica* and *R. ridibunda* occur syntopically in this region. SCHNEIDER et al. (1993) detected a considerable character displacement in several mating call parameters which maximize the differences of the mating call between the two species. SINSCH & EBLENKAMP (1994), in turn, detected specific differences in the genotypes of several enzymes in *R.*

Source MNHN, Paris

ridibunda of the Nestos region as compared to the genotypes from the brook at Valtos. This study has revealed the introgression of *R. ridibunda* genome into the *R. balcanica* genome at the Nestos River, e.g. allele b of the α-GPD and alleles b and c of the LDH-1. The alternatively fixed alleles of the ADA locus show that the occurrence of typical *R. ridibunda* alleles at other loci is due to introgression and not to an erroneous classification of some individuals of the Nestos samples. All these findings emphasize the importance of a further thorough study of the water frog populations in this area of distributional overlap between the two species.

Interspecific genetic differentiation

Our study clearly demonstrates that the chord distances between conspecific populations, even if they are separated by a large geographical distance, are always smaller than those between any interspecific pair. This genetic divergence holds also in the contact zone in the region around the Nestos River which apparently represents an ancient hybrid zone.

There are few estimates of Nei's distances between the three species of water frogs which were formerly referred to as *R. ridibunda* because they have only recently been distinguished: (1) *R. ridibunda* – *R. balcanica*: D = 0.082 (Sinsch & Eblenkamp, 1994); (2) *R. ridibunda* – *R. levantina*: D – 0.178 (Sinsch & Eblenkamp, 1994); (3) *R. balcanica* – *R. levantina*: D = 0.247 (Nevo & Filippucci, 1988) and D = 0.196 (Sinsch & Eblenkamp, 1994). All estimates of the genetic distances between the three species clearly fall into the normal range of genetic differentiation at species level in Amphibia (D = 0.1 – 3.0; Avise & Aquadro, 1982).

Protein analyses and bioacoustics

This investigation of the enzymatic and non-enzymatic proteins of the water frogs in northern Greece provides excellent corroboration of the results of previous bioacoustic analyses (Schneider & Sofianidou, 1985, 1986; Schneider & Sinsch, 1992; Schneider et al., 1984, 1993). It confirms both the presence of the two species *R. ridibunda* and *R. balcanica* and the local differences within *R. balcanica* (see figs. 2-3). The mating calls of *R. balcanica* in Epeiros have on average fewer pulse groups per call, and longer intervals between the pulses groups, than those of *R. balcanica* in Macedonia (Schneider & Sofianidou, 1986). But despite these local differences the mating calls of all populations are clearly classifiable as *R. balcanica* calls (Schneider et al., 1993).

In view of these protein analysis results, it seems desirable to continue both the bioacoustic and the electrophoretic studies in Greece, especially in the vicinity of the Nestos River and adjacent regions in Thrace and Macedonia, in order to determine where *R. ridibunda* and *R. balcanica* are sympatric and whether hybrids of the two species are present there. Hybrids have been found in other regions where two water frog species are sympatric: for example, hybrids of *R. ridibunda* and *R. lessonae* (Berger, 1964, 1973), of *R. ridibunda* and *R. perezi* (Graf et al., 1977), and of *R. balcanica* and *R. epeirotica*

Source MNHN, Paris

(SOFIANIDOU & SCHNEIDER, 1987). Therefore, it seems rather likely that hybrids of *R. ridibunda* and *R. balcanica* will eventually be found.

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Ecological observations on *Rana pretiosa* in western Utah

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The ecology of the spotted frog *Rana pretiosa* was studied in a small isolated marsh in western Utah (U. S. A.) during the spring of 1992. A total of 354 egg clusters were counted, averaging 444 eggs per cluster. The majority were laid in shallow water and were attached to other clusters, forming large communal masses. Eighty spotted frogs were marked and released, comprising 28 females, 25 males and 27 juveniles. Snout-vent length and weight of the females averaged 60.5 mm and 18.5 g respectively, of the males 51.6 mm and 11.0 g, and of the juveniles 33.3 mm and 2.2 g. Females were significantly larger and heavier than males. Population density was approximately 100 frogs per hectare. The rarity and highly isolated occurrence of spotted frogs in Utah, and the general decline of ranid frog species in western North America, call for protection of this population.

INTRODUCTION

Compared to other species of ranid frogs in North America, the natural history of the spotted frog *Rana pretiosa* is still only partially documented (TURNER, 1958, 1960, MORRIS & TANNER, 1969; LICHT, 1969, 1974, 1975). The spotted frog occurs predominantly in British Columbia, Washington, Oregon, northern Idaho, and western Montana, but also sporadically in Wyoming, Nevada and Utah, where the populations tend to be small and isolated. According to STEBBINS (1985), some populations of the spotted frog in Oregon and Washington are nearly extinct because of competition with leopard frogs and bullfrogs. DUMAS (1966) previously reported that the leopard frog is competitively dominant over the spotted frog and replaces it wherever they occur together. In Utah, the spotted frog is presently known from two areas, the west desert and along the Wasatch range (STEBBINS, 1985). In 1991, the United States Fish and Wildlife Service listed the spotted frog as a candidate (category 2) under the Endangered Species Act (Federal Register 56-225:58814). Recently, BARINAGA (1990) and PHILIPS (1990) pointed out world wide declines in amphibian populations. Similar declines have been recorded in several western North American frogs (CORN & FOGLEMAN, 1984, HAYES & JENNINGS, 1986; BRADFORD, 1991). Although TURNER (1958, 1960) conducted an extensive population study of the spotted frog in Yellowstone Park, and MORRIS & TANNER (1969) reported on the reproductive biology of a population from north central Utah (Provo), not a single life

Source MNHN, Paris

history account has been published from the extreme western populations of Utah, in particular from the Gandy Salt Marsh of Snake Valley. To date, the only evidence of spotted frogs at this site is observational. The present study was conducted to document the existence of the spotted frog in the Gandy Salt Marsh, and to assess its population structure and breeding dynamics.

Study area

The Gandy Salt Marsh is located at the southern end of Snake Valley between the ranching communities of Gandy and Trout Creek. The specific locality is at longitude 113° 55′ 13″ W and latitude 39° 28′ 48″ N. The marsh consists of several spring-fed ponds covering approximately 0 5 × 3.5 km, which drain eastward into an alkali lake bed (fig. 1). The substrate of the larger ponds is covered with a thick layer of muck, ranging in depth from about 30 cm to 1-2 m. The western edge of the marsh is flanked by an extensive mud flat, which is sparsely vegetated with salt grass (*Distichlis spicata*), grease wood (*Sarcobatus vermiculatus*), alkali rabbitbrush (*Chrysothamnus albidus*) and common reed grass (*Phragmites australis*). During spring, the mud flat is saturated and slick. The dominant vegetation surrounding the ponds (fig. 2) consists of bulrush (*Scirpus acutus*), cattails (*Typha*) and sedges (*Cyperus*) The dominant bottom vegetation consists of the stonewart alga (*Chara* sp.), common marestail (*Hippuris vulgaris*), filamentous green alga (*Spirogyra* sp.) and rush (*Juncus*). Most of the small, deep springs are covered with watercress (*Nasturtium officionale*). The study was conducted at the north end of the marsh, encompassing an area approximately 300 × 50 m with several small to medium sized ponds (Table I, fig. 1).

The virtual absence of native perennial grasses on the western boundary of the Gandy Salt Marsh, such as Indian rice (*Oryzopsis hymenoides*), galleta (*Hilaria jamesii*), needle (*Stipa comata*), and dropseed (*Sporobolus* sp.), indicates that this region has been heavily overgrazed (personal observation). This is further supported by the stunted growth and scarcity of the various native shrubs, such as greasewood (*Sarcobatus vermiculatus*), spiny horse brush (*Tetradymia spinosa*), shadscale (*Atriplex confertifolia*), snake weed (*Gutierrezia sorothrae*), big sage (*Artemesia tridentata*), indian tea (*Ephedra nevadensis*) and hop sage (*Grayia spinosa*). Most of the ground is exposed, trampled, and encrusted with alkaline deposits. The only ground cover are sporadic clumps of salt grass (*Distichlis spicata*), a halophytic species indicating high salinity. Because the western boundary slopes towards the marsh, salts are undoubtedly leaching into the ponds. Numerous cow trails lead directly into the marsh, enhancing the runoff and possibly the salinity. Although the ponds are continuously flushed by the springs, enough salts could accumulate over time to alter the freshwater invertebrate community, upon which the frogs depend for food.

Source MNHN, Paris

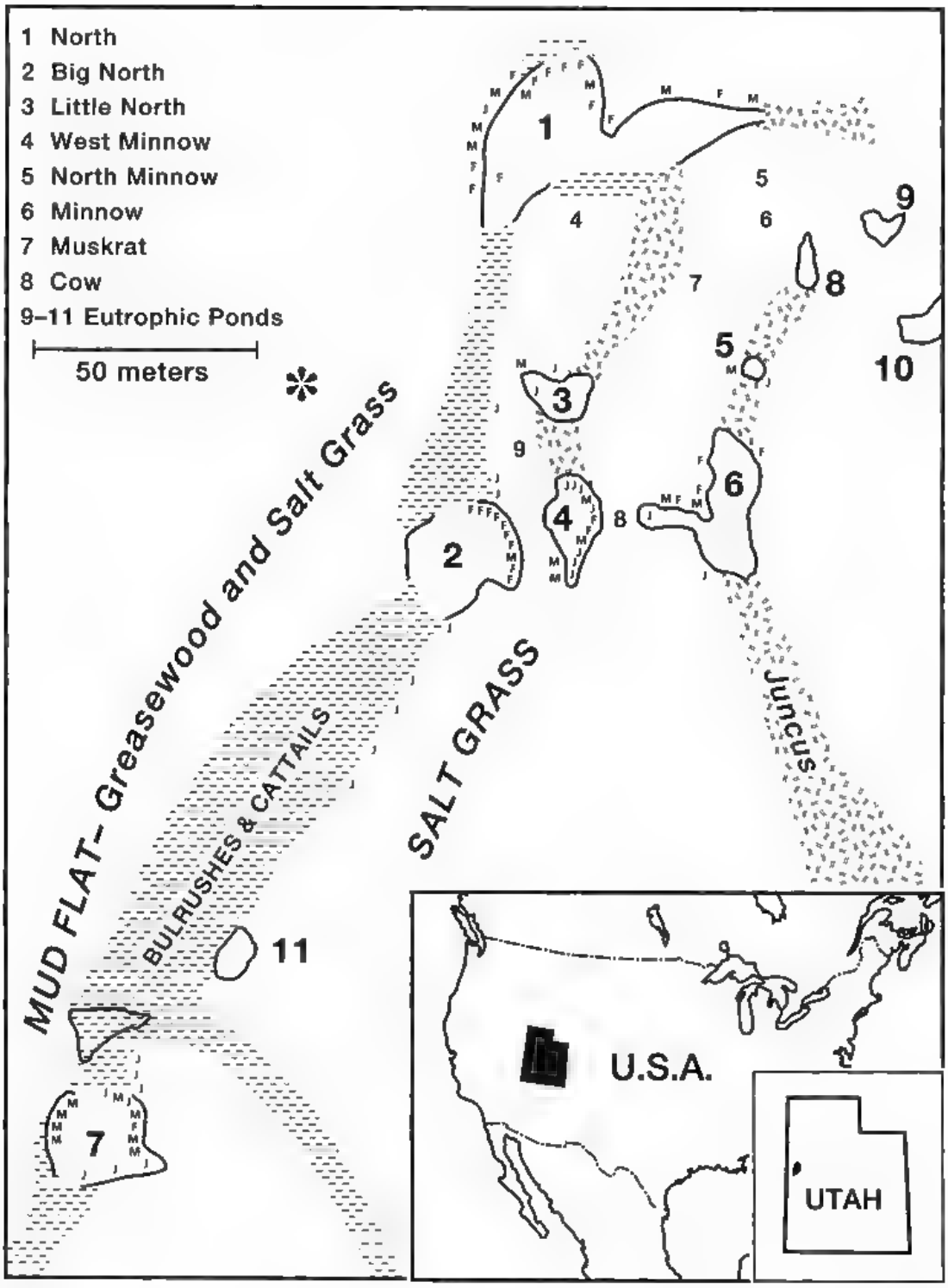

Fig. 1. – Generalized map of the study site (north end of the Gandy Salt Marsh) showing the various ponds described in the text, with inset of U.S.A. and State of Utah. The asterisk shows the location of U. S. Bureau of Land Management (BLM) Cadastral Survey, south west corner monument (1/4 S19 S30, T15S R18 W). Large numbers represent the ponds. Small numbers represent BLM metal stakes marking the location of spring heads. Letters represent the approximate site where each frog was collected. J: juvenile (yearling); F. female; M: male. Adults were collected predominantly along exposed banks.

Source MNHN, Paris

Fig. 2. – Southwest view of Big North Pond. Photo taken during the spring of 1993 Note ice along the edges and a flag marking the location of a frog captured the previous year. Mountains in the background are in the State of Nevada.

Fig. 3. Five recently laid egg clusters attached to each other and to the grass substrate. Note two male spotted frogs adjacent to the egg clusters. This photograph was taken during the spring of 1993.

Source MNHN, Paris

Table I. - The number and location of *Rana pretiosa* egg clusters observed. Most clusters were attached to substrate and to each other in masses Distance and depth in meters (depth = from water surface to bottom vegetation).

Pond	Date observed	No. of clusters	Shore distance	Cluster depth	Type of substrate
South	3/16	25	0.5	0.15	*Juncus*
South	3/16	4	1.5	0.15	*Juncus*
North	3/17	60	2.0	0.15	*Chara*
North	3/17	4	1.0	0.20	*Juncus*
Muskrat	3/17	8	0.3	0.10	*Juncus*
Muskrat	3/17	9	0.3	0.10	*Juncus*
Muskrat	3/17	25	2.0	0.20	*Typha*
Muskrat	3/17	35	3.0	0.30	*Typha*
Middle	3/20	85	2.0	0.40	*Juncus*
Middle	3/20	13	0.5	0.15	*Juncus*
Fenced	3/20	5	4.0	0.35	*Juncus*
Fenced	3/20	45	5.0	0.35	*Juncus*
E. Minnow	3/21	2	0.5	0.15	*Juncus*
N. Minnow	3/21	1	0.3	0.10	*Nasturtium*
Minnow	3/21	8	0.8	0.15	*Juncus*
East	3/21	5	0.5	0.15	*Juncus*
Little North	3/23	6	0.5	surface	*Juncus*
Little North	3/23	8	0.5	surface	*Juncus*
Little North	3/23	2	0.5	surface	*Juncus*
Big North	4/3	4	3.0	0.30	*Juncus*
		354	1.4	0.20	

MATERIALS AND METHODS

Field work was conducted from March 16 to April 10, 1992. All ponds were searched at least twice a day, mornings and afternoons. Captured frogs were individually marked by clipping one or two different toes, and were then released at the site of capture after recording sex, snout-vent length (SVL), weight, specific location and temperature. The site of initial capture was marked with a road-construction flag bearing the number and sex of the frog. SVL was measured from the ventral side. Weight was recorded with a spring scale (Pesola), and frog (cloaca), water and air temperature were measured with a digital thermometer. The location and number of egg clusters observed was also recorded and marked with flags All egg clusters in the marsh were counted. The number of eggs per cluster was estimated from 22 clusters by counting the number of eggs in a small sample of each cluster, weighing the sample, weighing the total cluster, multiplying the number of eggs in the small sample by the total weight of the cluster, and dividing by the weight of the small sample. Because spotted frogs lay their egg clusters together as a mass (fig. 3),

Source MNHN, Paris

and the clusters are intimately attached to each other, counting the exact number in the larger masses (> 45) was difficult. The southern end of the marsh system contains three large, somewhat interconnected ponds (Middle, South, Fenced), from which only eggs were enumerated (Table I).

Results

The only signs of mating activity detected during this study were sporadic choruses heard on March 16, 20, and 25. They were heard faintly from a distance, and the males stopped calling when approached to within 15-20 meters of the pond. A total of 354 egg clusters were counted during the study. Most were attached to other clusters, forming masses (fig. 3). The number of clusters in a mass averaged 17, ranging from 2 to 85 (Table I). Their average distance from shore was 1.4 m and their average depth from the surface was 0.20 m. The majority were laid over grass, to which they were firmly attached. Individual clusters were spherical, ranging in diameter from about 6 to 12 cm. The number of eggs per cluster averaged 444, ranging from 325 to 710. Most masses contained clusters in different stages of embryonic development. The average weight of clusters with embryos was significantly greater than the weight of clusters with recently laid eggs, 157 g and 113 g respectively ($t = 2.3$, $df = 20$, $P < 0.05$), but both averaged the same number of embryos, 441 and 421 respectively. Recently laid eggs were approximately 2 mm in diameter. Each egg was surrounded by two clear gelatinous capsules, the outer one about 12 mm in diameter, and the inner one about 6 mm. Tail bud embryos were about 3 mm in length. Hatching was first observed on March 25, with total length of the emerging larvae about 8-10 mm. On April 6, 36 tadpoles measured at North pond averaged 13.4 mm (range = 11-15 mm, SD 1.5). A sample of thirty-two (all 15 mm long) weighed 1.9 g, averaging 0 06 g / tadpole. By this time, most had dispersed from the masses to the grassy shoreline. Ten laboratory reared tadpoles weighed on May 19 averaged 1.98 g / tadpole (range = 1.55-3.05 g). Their bodies averaged 24.5 mm (range = 20-25) and tails 31.4 mm (range = 25-35). All had small developing rear legs.

Eighty spotted frogs were marked and released during the study, consisting of 28 females (35 %), 25 males (31 %) and 27 juveniles (34 %). The females averaged 60 5 mm snout-vent length (range = 51-70, SD = 5.7), the males 51.6 mm (range = 45-59, SD = 3.0) and the juveniles 33.3 mm (range = 26-40, SD = 3.3) (fig. 6). The females averaged 18 5 g in weight (range = 12-28, SD = 4.2), the males 11.0 g (range = 7-15.3, SD = 1.6) and the juveniles 2.2 g (range = 1.6-5.4, SD = 1.1). The females were significantly larger and heavier than the males (SVL: $t = 7.24$, $P < .001$, WT: $t = 10.3$, $P < .001$). The smallest size of a mature male was 45 mm. Mature males had distinctly dark and swollen nuptial pads on the thumbs. The dorsal coloration of both sexes was greenish brown with faint dark spots (figs. 3-4) and the under surface was yellowish, especially in the axillary and inguinal areas.

Lincoln-Peterson estimates conducted during the last five days of study averaged a total population size of 146 frogs in the north marsh. This area covers approximately 1.5 hectares, so the total population density is about 100 frogs per hectare, 1/3 each females,

Source MNHN, Paris

Fig. 4. — An amplectant pair of spotted frogs from North Pond during early March of 1993

Fig. 5. — A floating mass of eggs with the surface exposed to the air. Note bulging bubbles and whitish, expanded capsules.

Source MNHN, Paris

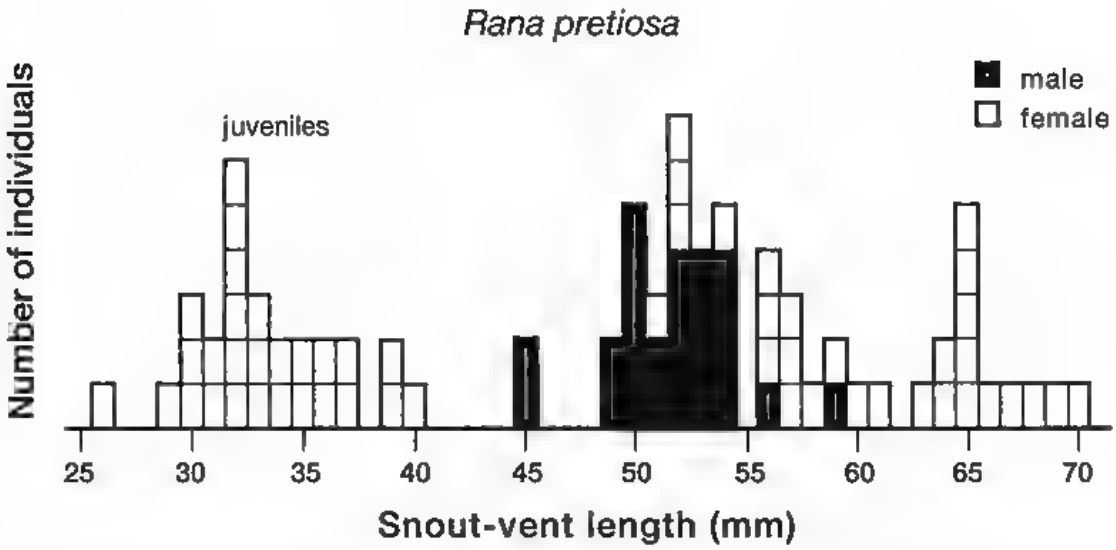

Fig 6. — Snout-vent length distribution of the juveniles and adult male and female *Rana pretiosa*.

males and juveniles. Most of the adults were captured in the large ponds, whereas most of the juveniles were captured in the small, grassy ponds and sloughs. The majority of frogs were detected on shore, partly immersed in the water and concealed under the bank vegetation. Of the 80 marked frogs, 24 (30 %) were recaptured once (Table II), 6 twice, 2 three times and 2 four times. Of the 24, 16 (67 %) were recaptured within 4 meters of their original capture site, and of these, 6 were recaptured at the same site. Two females (5 and 7) were recaptured three times at the same site. The average distance traveled away from the point of first capture by females was 25 m, by males 14 m and by juveniles 0.9 m (Table II). The longest distances traveled from the original capture site were 100 m, 150 m and 3 m, respectively. One male and one female migrated to other ponds. Mean body temperatures of adult frogs were consistently higher than both water and air, about 18.4 compared to 15.8 and 14.9, respectively.

Discussion

The virtual absence of calls after March 18-25 suggests that most of the breeding occurred during early March. Because the majority of the egg clusters were found at the beginning of the study, and some of the eggs had developed to the tail bud stage, oviposition probably occurred several days prior to the study. The most recently laid clusters were smaller (about 8 cm) than those with tail bud embryos (about 10-12 cm), suggesting that the jelly capsules expand as development progresses. Turner (1958) and Morris & Tanner (1969) report that the expansion is due to water absorption. Indeed, clusters with embryos were significantly heavier than those with eggs, even though the number of embryos and eggs was similar.

Source MNHN, Paris

Table II. - Number of recaptures and total distance *Rana pretiosa* traveled (meters) from original point of capture. All recaptures represent different days. 0 = same place of capture.

Frog No	Frog Sex	Locality	No. of recaptures	Distance and direction from first capture 1	2	3	4	Total distance traveled
1	F	Big North	4	0	25S	25S	20N	70
4	F	Minnow	1	8E				8
5	F	North	4	2S	2S	30S	2S	58
7	F	North	3	2W	2W	2W		2
10	F	Minnow	2	12W	2E			26
12	F	West Minnow	2	4S	17S			17
16	F	Big North	1	100N	Moved to N. Pond			100
17	F	Minnow	1	3N				3
18	F	North	1	2N				2
1-5	F	North	1	3W				3
1-13	F	West Minnow	1	14S				14
1	M	Minnow	1	75N	Moved to Linkage			75
8	M	Minnow	1	5				5
10	M	West Minnow	1	4W				4
11	M	West Minnow	2	3N	6N			6
13	M	Minnow	1	13E				13
15	M	Minnow	1	5				5
17	M	Minnow	1	3S				3
2-7	M	North	1	19E				19
11	J	Minnow	1	22N				22
1-18	J	Big North	1	5				5
2-6	J	Minnow	1	3S				3
2-11	J	Minnow	1	0				0
2-18	J	Little North	1	0				0

In addition to expanding in volume as development proceeds, the clusters begin rising to the surface and the inner capsules develop a population of algae, giving the clusters a greenish appearance (see SVIHLA, 1935; MORRIS & TANNER, 1969). By hatching time, the entire mass floats to the surface, where it spreads and assumes a frothy, yellowish-green texture (fig. 5). The expanding bubbles force many larvae to the surface where they die from dehydration. According to MORRIS & TANNER (1969), 10 to 20 percent of surface eggs are destroyed this way The bubbles probably develop from oxygen produced by the algae. After hatching the free-swimming larvae remain below the egg mass, attached by their mouths to the capsules, possibly feeding on the algae. The floating mass also apparently serves as camouflage for the developing larvae, and may also enhance the speed of development by increasing water temperature at the surface. The surface temperature of one mass at Little North Pond was 24°C compared to 10°C at the bottom of the pond.

Source MNHN, Paris

The fact that the majority of egg clusters were deposited together in masses suggests a communal type of breeding in which several gravid females are attracted to the same place, perhaps by several males calling together. Similar communal breeding was reported by TURNER (1958), LICHT (1969), and MORRIS & TANNER (1969). Presumably, each egg cluster represents a single clutch, but the exact number of clutches deposited by each female has not been documented. The northern ponds contained 177 clusters (Table I), but the number of females actually captured was 28. Even the Lincoln-Peterson estimate of 51 females corresponds to less than one third of the clusters. Therefore, either the population is much larger than currently estimated, or some females lay more than one cluster.

The wide variation in number of eggs per cluster reported here, ranging from 325 to 710, suggests that some females may have split their clutches into two or more smaller clusters. Other workers have also reported wide variation in egg numbers in the spotted frog (TURNER, 1958: 206-802; LICHT, 1969. 249-935; MORRIS & TURNER, 1969: 148-1160). Some of this variation could be related to differences in the size and age of females, because female body size is positively correlated with clutch size in most frogs (PETTUS & ANGLETON, 1967; SALTHE & DUELLMAN, 1973; BERVEN, 1982). *Rana sylvatica* is known to deposit two or more egg masses in one breeding season (DAVIS & FOLKERTS, 1986). The current estimate of 51 females in the north ponds suggests that each female on average may lay 3.5 egg clusters (177/51).

Judging from the small number of frogs captured daily, only a fraction of the total population appears to be active at one time. Moreover, spotted frogs at this site are highly secretive and elusive. When approached, they usually slip into the water quietly, leaving only a small swirl. Of the 80 captured frogs, only four were observed entirely out of the water, basking on grass beneath the bank. Even so, the majority had a higher body temperature than the water, suggesting they are only partly submerged in their perches. Because most of the frogs were recaptured very close to the original site of capture, they apparently use the same perches repeatedly, suggesting they are highly sedentary. Although the overall sex ratio was nearly equal, some ponds had a predominance of one sex (Table III). The only other anuran observed in this marsh was the northern leopard frog, *Rana pipiens*. Six individuals were found at the extreme south end, one pair in amplexus. Leopard frogs are apparently rare in this marsh, or emerge much later. They also seem to prefer the larger ponds at the southern end. The northern end is inhabited exclusively by spotted frogs. However, the abundance of spotted frog eggs in the southern ponds indicates that they breed throughout the marsh system. At this time, leopard frogs at this site do not seem to be replacing the spotted frog, as has been reported previously by DUMAS (1964). During the spring of 1993, however, I observed a single adult leopard frog about 10 meters southeast of Muskrat pond.

The presence of cow dung in many of the ponds, especially the shallow ones, indicates that cattle traverse them, trampling the aquatic vegetation, and possibly the frogs themselves. Since the ponds provide the most luxuriant foliage in this region, and the only source of fresh water, cattle tend to congregate around them, grazing the succulent pasture The highest concentration of cow dung and trails is along the banks, the critical feeding, breeding and basking habitat of the frogs. Several ponds at the north end contain a dark-reddish water, seemingly from dung eutrophication. One of these ponds ("Cow

Source MNHN, Paris

Table III. - The number of female, male and juvenile *Rana pretiosa* captured per pond.

Pond	Females	Males	Juveniles
North	12	6	1
Little North	0	1	2
Big North	9	1	2
West Minnow	2	4	7
North Minnow	0	1	1
Minnow	4	2	2
Muskrat	1	8	8
Linkage	0	0	6

Pond") is littered with bones from a dead cow, which apparently died within it. All such ponds are devoid of aquatic vegetation, invertebrates and frogs. The remains of a single dead cow in a fertile pond would undoubtedly destroy its entire frog population. A major ranching problem in this region (Snake Valley) is the frequent drowning of cattle in the deeper ponds, which are usually fenced to prevent miring (Terry HALE, pers. comm.).

Data from this study suggest that the population of spotted frogs at the Gandy Salt Marsh is small, perhaps comprising less than three hundred individuals. The marsh itself also is very small, consisting of a few isolated springs within a vast arid valley. According to BEGON et al. (1990), when local populations are reduced to "several hundred" individuals, they are prone to becoming extinct because of localized catastrophes, such as drought. They also list the major causes of rarity according to the International Union for the Conservation of Nature and Natural Resources. Most of these causes of rarity and extinction apply to the ecology and distribution of the spotted frog in western Utah. Its habitat is rare (isolated fresh water springs). Its resources are limited (confined to a few spring-fed ponds). It cannot disperse to other similar habitats, and it is limited by cattle, which degrade its aquatic habitat and trample its breeding and basking sites In the interest of protecting this vulnerable endemic species, livestock grazing should be discontinued from the western boundary of the marsh, the principal watershed. This would allow repopulation of the native grasses and shrubs, reducing erosion, evaporation, and salinization.

RESUMEN

La ecología de la rana pinta *Rana pretiosa* fue estudiada en varios lagitos aislados en la parte norteña de la cienega salada de Gandy en la frontera occidental de Utah durante la primavera de 1992 Un total de 354 nidadas fueron contadas con un promedio de 444 huevos por nidada. La mayoría fueron puestas a orillas de lago pegadas con otras, formando masas comunales. Un total de 80 ranas fueron marcadas y soltadas consistiendo de 28 hembras, 25 machos y, 27 añeras. El promedio del tamaño (hocico-cloacal) y peso

Source MNHN, Paris

de hembras fueron 60.5 mm y 18.5 g, respectivamente de machos 51.6 mm y 11.0 g, y de añeras 33.3 mm y 2.2 g. Hembras son significativamente más grandes y más pesadas que machos. Densidad poblacional es aproximadamente 100 ranas por hectarea. Debido a la rareza y distribución muy aislada de esta rana en Norte América debería protejerse la población en Gandy de su posible extinción.

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Défense et illustration de l'histoire naturelle avec Muséum 2000

Jamais époque n'a connu atteinte aussi grave à l'encontre de la nature: extinctions sans cesse plus nombreuses d'espèces animales et végétales, pollution des eaux, altération de l'atmosphère, dégradation de la pellicule pédologique, auxquels s'ajoutent appauvrissement des cultures et malaise généralisé de l'humanité. Ces drames sont le plus souvent des retombées d'une évolution scientifique, technologique, démographique foisonnante et mal maîtrisée. Face à eux, le citoyen pourrait penser qu'il s'impose de privilégier les études sur cette nature - sur tous les aspects de cette nature - ainsi mise à mal. Or, et ce n'est pas le moindre paradoxe de notre époque, en France tout spécialement, l'histoire naturelle est la mal-aimée des pouvoirs publics et des technocrates qui les orientent. A tous les stades de l'enseignement aussi bien que dans la recherche, les sciences naturelles régressent, l'observation est méprisée, l'approche globale est ignorée, le tout au profit de disciplines et de techniques plus "modernes": or n'est-il pas évident que ces dernières - dont l'intérêt est immense - ne sont plus rien si les objets de leur étude comme l'interprétation de leurs résultats ne s'insèrent pas dans le cadre que seules peuvent leur apporter les disciplines naturalistes de base...

Depuis des siècles, le Muséum national est en France le bastion de l'histoire naturelle Il est à ce titre depuis quelques années la cible de l'hostilité tenace et destructrice des contempteurs de nos disciplines. L'association Muséum 2000 (association sous la loi de 1901, fondée le 29 novembre 1990) s'est fixé pour objectif de défendre cet établissement prestigieux et irremplaçable. Cette vocation implique une volonté inébranlable de défendre l'histoire naturelle dans notre pays et hors de celui-ci. Cette association est ouverte à tous Nous faisons appel à tous ceux que peut motiver notre idéal, chercheurs, enseignants, professionnels ou amateurs, citoyens sensibles au drame de notre temps, pour qu'ils nous apportent leur soutien, leur concours, leur dynamisme, leur adhésion

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The problem of declining amphibian populations in the Commonwealth of Independent States and adjacent territories

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Published evidence of amphibian declines within the territory of C.I.S. and adjacent territories (formerly U.S.S.R.) are reviewed. Populations of more than half of all the species are subject to decline. Most of the recorded declines and extinctions are local; the main causes are anthropogenic factors, especially deforestation, destruction of water bodies, pollution and urbanization. Few species are expanding under anthropogenic influences. The history of the expansion of *Rana ridibunda* is briefly discussed.

INTRODUCTION

The study on amphibian ecology in the former U.S.S.R. has been conducted for several decades. The recent dismemberment of the U.S.S.R. has led to the formation of the Commonwealth of Independent States (C.I.S.) and few republics have not joined this.

Here I summarize the published and some unpublished data on the phenomenon of declining amphibian populations within this territory. Analysing such information, the researcher faces several problems. How to distinguish between stable declines and temporary fluctuations in numbers? What are the extents of declines? Which conclusions on the declines were made on the basis of subjective estimations, and which on the basis of exact quantified data? Unfortunately, not all the papers reviewed contained the answers to these questions. However, a comparative analysis permits some conclusions on the population trends in different regions. In the analysis I have used puplications concerned with almost all the republics of the former U.S.S.R. Papers with data only on population number and lacking data on dynamics were not used.

Generally, the population changes may be analysed on two time-scales: long-term and short-term. The first includes, conventionally, changes in geological and historical times: covering millions to hundreds of years. The second includes changes from a century to one year or less, and concerns fluctuations in population numbers.

Source MNHN, Paris

Long-term changes

One can say with certainty that global changes of climate have influenced amphibian numbers and distributions. A large proportion of contemporary Eurasian amphibian species already existed in the late Pliocene. Glaciations and regressions of glaciers, expansions and reductions of forests and steppes during the last 600-700 thousand years had a great impact on the distribution and dynamics of amphibians (Borkin, 1984). For example, Quaternary remains of a toad related to *Bufo raddei* (now representative of the Mongolian fauna) were found in European Russia. Quaternary bones resembling those of *Salamandrella keyserlingii* have been found c. 800 km SW from the species' contemporary range (Ratnikov, 1989). In addition, many bones of Quaternary tailless amphibians attributed to extinct species have been found in Russia. Thus, we can ascertain the extinctions of amphibian species within the territory of former U.S S.R. during the last few hundred thousand years.

It is evident that more recent changes have also occurred. For example, changes have probably taken place during the last thousand years in the territory of Tatarstan (Garanin, 1989). During the cold periods the northern taiga species *S. keyserlingii* occurred there and, in warm periods, *Hyla arborea*. Forest species, *Triturus cristatus*, *Bufo bufo*, and *Rana temporaria*, receded northwards due to forest destruction. In the Vth-XIIIth centuries and later, the increase of agriculture may have caused the spreading northwards of the southern species *Bombina bombina* and *Pelobates fuscus*. Thus, long-term changes in climate and landscape may have influenced species in different ways, according to their specific ecological requirements.

Short-term changes

Population declines

Taxa

From 13 species of Caudata inhabiting the former U.S.S.R., declines are documented in 11 (Table I). The remaining two, *Triturus dobrogicus* and *Hynobius turkestanicus* are simply unexplored. From 27 anuran species, declines are documented in 15 (Table I). This probably reflects the better state of anuran populations compared to caudates, rather than the scarcity of information on some anuran species. Most reports concern the declines of widespread and numerous species, such as common toad, brown frogs, etc. The declines of endemic and relic species (*O. fischeri*, *R. sibiricus*, *M. caucasica*, *T. vittatus*, *T. montandoni*, *T. alpestris*, *T. karelini*, *P. caucasicus*, *P syriacus*) are probably local. Nevertheless, such declines may have severe consequences due to low total number and narrow ranges.

Source MNHN, Paris

Time and regions

Most data on declines are from the period 1970-1990. Basically, this reflects increasing interest in nature conservation at this time. At the same time, there was an increase in anthropogenic influences on amphibian populations. Earlier declines in the 1920s and 1940s were connected mainly with the destruction of habitats due to urbanization, industrialization, establishment of collective farms, and with the Second World War (BANNIKOV & ISAKOV, 1967; BESKROVNY & BURMENSKAYA, 1970).

The most numerous declines have been registered in the European region (Table I). This reflects not only the better exploration of this region, but probably the real situation. Reports from Western Siberia and the Far East are much less numerous, though these regions have been studied to a reasonable extent.

Natural factors

Natural factors (see legend of Table I) are rarely reported as causes of amphibian declines. In these instances the main factor is probably natural community succession leading to eutrophication and overgrowth of the breeding ponds. These factors are indicated for four amphibian species, and may have led to extinctions of isolated populations especially in urban areas.

Anthropogenic factors

Anthropogenic factors probably play the main role in the recent declines (see Table I). *Destruction of forests and other arboreal vegetation* appears to be most widespread. This factor is indicated in 23 % of papers containing information on the causes of declines. This leads not only to destruction of terrestrial habitats, but also to the drying of breeding ponds and brooks. Deforestation was indicated as the cause of declines of 40 % of species studied. This is most harmful for the forest species such as newts and common toads. *Pond drainage* has been reported as a second main factor of amphibian declines (20 % of papers; indicated for 44 % of species). *Intensive use of artificial fertilizers and pesticides* was reported as a factor of declines in 14 % of reports and for 40 % of species. This influences amphibians mainly by spoiling of water, frequenly caused by *water pollution by cattle and industrial wastes* (each reported in 11 % of papers). Nevertheless, the second kind of pollution appears to be more harmful than the first: this was reported for 40 and 16 % of species studied, respectively. Other anthropogenic factors (see footnote to Table I) have been reported relatively rarely (in 3-6 % of papers) and concerned, as a rule, only 4 to 13 % of species studied.

Only *urbanization*, as a complex factor, being not frequently indicated, concerns 36 % of species. This has caused sharp declines of amphibians In the large cities, such as Moscow, Nizhny Novgorod and Ekaterinburg, amphibian numbers decreased from the periphery of city to the centre (BANNIKOV & ISAKOV, 1967; LEBEDINSKY, 1981; VERSHININ, 1987; KUZMIN, 1989; USHAKOV & BELOBORODOVA, 1989). *B. viridis*, *R. temporaria*, *R. arvalis* and *R. ridibunda* are better adapted to life in urban areas than other amphibians, but under excessive urbanization they also have disappeared. The process of extinction

Source MNHN, Paris

Table I - Amphibian declines in the territory of former U S S R

Years —: unknown

Causes of declines - A. anthropogenic factors (1 destruction of forests and other arboreal vegetation, 2 destruction of hiding places, 3 streambank damage by cattle; 4 intensive cattle pasture, 5 intensive use of artificial fertilizers and pesticides in agriculture, 6 use of pesticides, 7 use of fertilizers, 8 removal of the rice fields, 9 earthwork, 10 pond drainage, 11 pond destruction, 12 pond clearing and building of embankments, 13 water pollution by cattle; 14 industrial pollution of water; 15 pollution of water by domestic wastes, 16 transportation of logs by heavy traffic; 17 overflooding; 18. pollution and drying of streams, 19. trailing of the cut trees along the streams, 20 river bank clearing and building of embankments, 21 introduction of the fish *Percottus glehni*, 22 introduction of *Rana ridibunda*, 23 urbanization, 24. collecting for commercial aims, 25 collecting for educational aims, 26 collecting for scientific aims, 27 fishing, 28 intensive recreation), N natural factors (1. mudding of ponds; 2 partial drying of ponds, 3 increase of water eutrophication, 4 overgrowth and shallowing of ponds, 5 probable increase of temperature and decrease of humidity), — unknown

Sources of information. - 1-63 numbers of references in "Literature cited", SK personal unpublished data, RK personal communication of R. A. Kubykin

Species	Regions	Years	Causes of declines	Sources of information
Salamandrella keyserlingii	Siberia Upper Angara River	—	A2, A10	42
Onychodactylus fischeri	Far East	—	A1	46
Ranodon sibiricus	Kazakhstan Upper Cherkassai River	1970s	A26	34
	Kazakhstan Dzhungarian Alatau		A3, A13, A27	14
Salamandra salamandra	Ukrainian Carpathians	1960-1970s	A1, A24, A25	43, 52
Mertensiella caucasica	Georgia	—	A18	26
	Georgia	1980s	A19	SK
Triturus vulgaris	Moscow	1920-1980s	A11, A12, A14, A23	SK, 10, 36
	Moscow Province: Glubokoe Lake	1973-1990	A21	38
	Volga-Kama region	—	A1	19
	Lower Volga	1973-1988	A5	33
	Ukrainian Carpathians	—	A6, A10, A14, A15	54
	Azerbaijan	—	A8, A10	1
Triturus vittatus	North Caucasus	1960-1980s	A7, A10, A13, N1, N2, N3	57
	Georgia	—	A10, A13, A14	26
Triturus montandoni	Ukrainian Carpathians	10-15 years	A24, A25	43, 54
	Skobelevskie Beskidy, Lvov	1948-1984	A16	52, 53
Triturus alpestris	Ukrainian Carpathians and Skobelevskie Beskidy, Lvov	10-15 years	A24, A25	43, 54
Triturus karelini	Chechnya	—	A1	3
	Azerbaijan	—	A10	1
Triturus cristatus	Moscow	1980s	A10, A11, A14, A23, N3, N4	SK, 10, 36
	Moscow Province: Glubokoe Lake	1973-1990	A21	38
	Volga-Kama District	—	A1	19
	Delta of Don	1920-1945	A1	12
	Ukrainian Carpathians		A6, A10, A14, A15	53

Source : MNHN, Paris

Table I. - Continuation.

Species	Regions	Years	Causes of declines	Sources of information
Bombina bombina	Moscow	1920-1980s	A10, A11, A23	10, 36
	Volga-Kama region	—	A1	19
	Severny Donets	1980-1986	A13	21
	Lower Volga	1973-1988	A5	33
	Ukrainian Polesje	—	A10	63
Pelodytes caucasicus	North Caucasus	—	A1, A9, A18	22
	Chechnya	—	A18	3
	Georgia	—	A18	26
Pelobates fuscus	Moscow	1922-1966	A11, A23	10, 36
	Moscow Province	—	A28	23
	Lower Volga	1973-1988	A5	33
	Severny Donets	1980-1986	A4	21
	Steppe on Dnepr	—	A14	39
Pelobates syriacus	Georgia	—	A10, A13, A14	26
	Armenia	—	—	16
Bufo bufo	Moscow	1922-1966	A11, A23	10, 36
	Voronezh	1950-1970	A1	19
	Volga-Kama region	—	A1	19
	Novosibirsk	1939-1969	—	54
	Altai Mountains	1980s	A22	62
	Baikal region	1960-1970	A1, A4	49
	Steppe on Dnepr and Dnestr Rivers	—	A1, A5	30, 51
Bufo viridis	Moscow	1922-1980	A10, A11, A14, A23	SK, 10, 36, 40
	Severny Donets	1980-1986	A4	21
	Lower Volga	1973-1988	A5	33
	Tbilisi	—	A2, A6	28
Hyla arborea	Steppe on Dnepr	—	A5	30
	Ukrainian Carpathians	—	A1	43
Hyla japonica	Siberia Zea River	—	A17	25
Rana temporaria	Moscow	1922-1980	A11, A14, A23, N4	SK, 10, 36
	Moscow Province	—	A6, A7	40
Rana arvalis	Moscow	1922-1980s	A11, A14, A23, N4	SK, 10, 36
	Moscow Province	—	A6, A7	40
	Serveny Donets	1980-1986	A4	21
	Volga-Kama region	1953-1963	N5	18
	Lower Volga	1973-1988	A5	33
	Novosibirsk	1939-1969	—	54
	Altai Mountains	1980s	A22	62
Rana macrocnemis	Turkmenistan Kopetdag Mountains	—	A1	6
Rana asiatica	Kazakhstan Kapchagai District	1960s-1970s	A17	RK
Rana lessonae / esculenta	Moscow	1920-1980	A11, A14, A23, N3, N4	SK, 10, 36
	Ukrainian Polesje	—	A10	63
Rana ridibunda	Moscow	1920-1980	A11, A14, A20	SK, 10, 36
	Lower Volga	1973-1988	A5	33
	Ukrainian Polesje	—	A10	63
	Kyzgyzstan Chu River	—	A25, A26	58

Source : MNHN, Paris

starts from the fragmentation of a population by residential districts and main roads. Then, as a result of isolation, susceptibility to anthropogenic and natural influences (inbreeding, droughts, frosts, pollution, etc.) is increased. After the last breeding centres have been destroyed, the animals may breed for a some time in shallow puddles. Finally, the population becomes extinct after the last adults die. Population declines may occur even if some ponds remain, if they have been cleared and concreted. The latter make the shores unsuitable for most amphibians.

The *influence of reservoirs* is complex. Commonly these did not lead to sharp changes in amphibian species composition Some species (mainly terrestrial) may decrease in number, but some time later (up to 10 years) they can recover (KALETSKAYA, 1953; ILYASHENKO, 1989; SMIRNOVA & EGOROV, 1985, DERKACH et al, 1989; USHAKOV & PISARENKO, 1989). In the Carpathian Mountains, the construction of reservoirs permitted some lowland species to enter the mountains, such as *T. vulgaris*, *T. cristatus*, *R. ridibunda*, *R. lessonae/esculenta* (POLUSHINA, 1977)

Extent

The extent of amphibian declines varies among the species and is determined by different causes. Local extinction, as a rule, is caused by a complex of anthropogenic factors. These situations were indicated for all the species listed in Table I, with a few exceptions: *S. keyserlingii*, *T. karelini*, *H japonica*, and *R. macrocnemis*. Local extinctions are most frequent in European region, reflecting the greater extent to which it has been studied. Deforestation is the most frequent key factor in local extinctions (POLUSHINA, 1977; GARANIN, 1983). However, in a historical perspective, this may have more "global" consequences for species (change of geographical ranges, etc.). Urbanization leads to local declines. However, the rate of these declines is species-specific. In general, forest species tended to decline and become extinct more rapidly than open-area species.

Some factors are reported to cause declines but not extinction For example, introduction of the fish *Percottus glehni* into some ponds in the Moscow Province led to local declines of *T vulgaris* and *T. cristatus* to less than 10 % of their previous abundance (MANTEIFEL et al , 1991) Intensive cattle pasture led to marked declines in *B. bombina* and *R. arvalis* numbers in different habitats of Severny Donets River basin (European Russia) (GOGOLEVA, 1987).

Some factors have had complex negative influences. For example, felling of trees and their trailing along the moutain streams led to almost total extinction of some Georgian micropopulations of *M. caucasica* (personal observations). On the other hand, on the Carpathian Mountains timber transportation by heavy traffic leads to the formation of deep ruts in the roads, creating suitable conditions for newt breeding. The newts are attracted by newly established water bodies and breed there. This led to the killing of *T. montandoni* and *T. alpestris* (mainly eggs and larvae) by traffic and drying As a result, the population declined markedly (TARASZCZUK, 1985). Although overall amphibian mortality on the roads may be high, its influence on population declines is unclear (GANEEV et al, 1985; RYZHEVICH, 1989).

Source MNHN, Paris

FLUCTUATIONS IN NUMBERS

Changes in population sizes have been documented in several regions of the former U.S.S.R. Fluctuations caused by anthropogenic factors were briefly noted above. Fluctuations of the numbers of *R temporaria* and *R arvalis* are caused by periodic droughts and frosts (SERGEEV & VETSHEVA, 1942; BANNIKOV, 1948; KALETSKAYA, 1953). After droughts marked reductions in the numbers of brown frogs occur, caused by breeding ponds drying up. Afterwards frog numbers recover due to successful breeding in renewed ponds. Fall in population size is also caused by high mortality in hibernacula during frosty winters with little snow. Different species show different reactions to both factors. *R. temporaria* is less tolerant of drought than the more southerly *R arvalis*. The latter, however, is less tolerant of frosts due to its exclusively terrestrial hibernation. Green frogs living in permanent waters express higher tolerance to both factors. Monitoring of amphibian populations in Volga-Kama region has shown wide annual fluctuations of both specific and total amphibian densities (sometimes by 20-30 times) against the background of the total decline (fig. 1). In other cases, the fluctuations are not accompanied by this trend (fig. 2).

POPULATION INCREASE AND DISPERSAL

Extensions of geographical ranges and population increases may occur not only in geological time, but also during the short term. For example, in Belorussia the increase of *P. fuscus*, *B. calamita* and *H. arborea* probably took place in the XXth century (SAPOZHENKOV, 1961). Its causes are still unknown. In Moscow Province the increase in number and the dispersal of southern species (*B. bombina*, *P fuscus*, *B. viridis*, *R. arvalis*) occurred in the 1920-1940s, whereas the northern species either reduced their numbers (*B. bufo*), or remained almost unchanged (*R. temporaria*) (BANNIKOV,1955). As in *B bufo*, *B. viridis* and *R. arvalis*, this tendency was observed at least up to the 1980s (personal observations). This is probably related to the general warming of the regional climate.

However, population increases and dispersals are basically related to anthropogenic factors. In Moscow Province ploughing up the water meadows led to a local increase of *P. fuscus* (GORBUNOV, 1989). Tree-felling and country-road building in forests resulted in the formation of small artificial pond systems and promoted the increase and local dispersal of *S. keyserlingii*, *P. fuscus*, *B. bufo*, *B viridis*, *R. temporaria* and *R. arvalis* (POLUSHINA, 1977; PIKULIK, 1985; KUTENKOV, 1990; ISHCHENKO, personal communication). These were documented in Karelia, Ural, Belorussia and Carpathians. The creation of artificial water bodies (fishponds, channels, sedimentation reservoirs, etc.) has promoted population increases in *S. keyserlingii*, *T. vulgaris*, *T. vittatus*, *B. bombina*, *P. fuscus*, *B. bufo*, *H. arborea*, *R. arvalis*, *R. temporaria*, *R. ridibunda*, and *R. lessonae/esculenta* (TOPORKOVA, 1977; KUBANTSEV & ZHUKOVA, 1981, KIREEV, 1983; TUNIYEV et al., 1986; GOGOLEVA, 1987; TARASZCZUK, 1987; KUTENKOV, 1990; personal observations). These events were observed in Central and Southern Russia, Kalmykia, Karelia, Middle Ural, Northern Caucasus, forest and steppe zones of the Ukraine.

Source MNHN, Paris

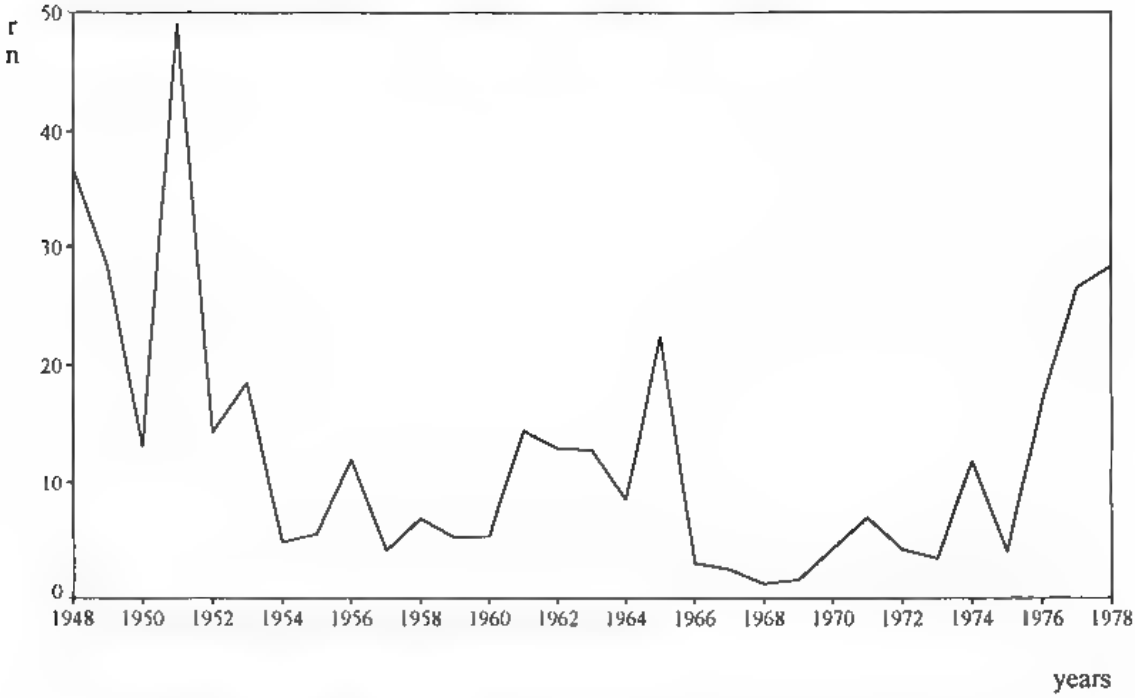

Fig.1. – Annual fluctuations in amphibian numbers in a plot of Volga-Kama region, Russia (from GARANIN, 1983)

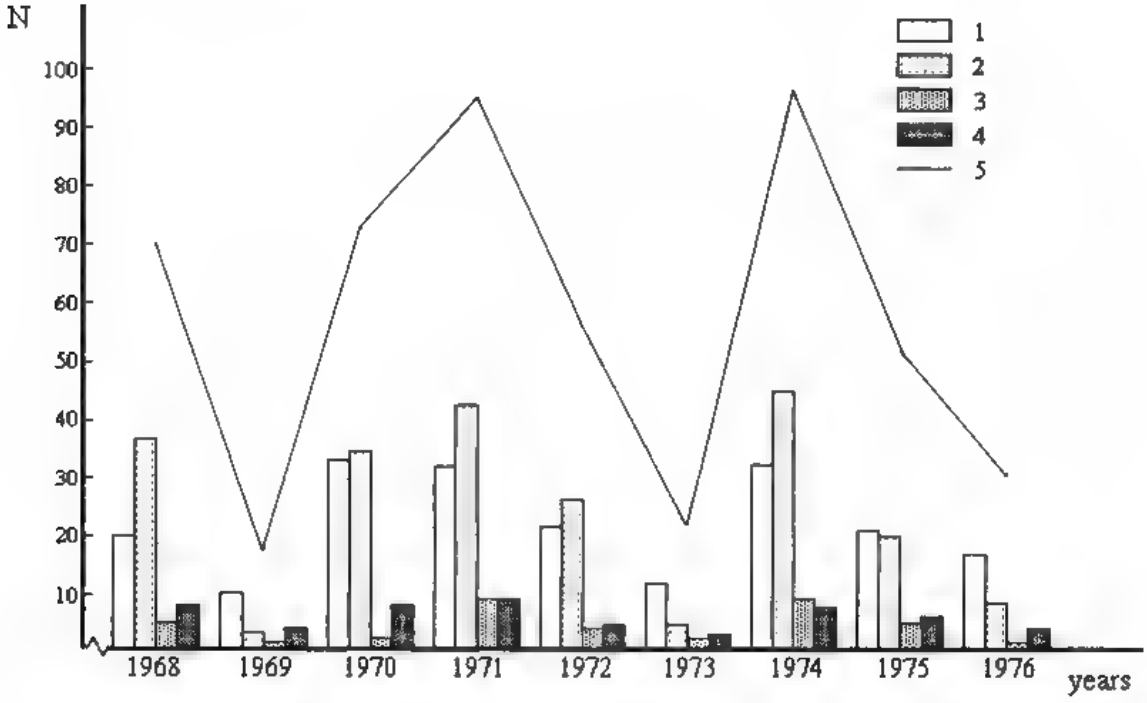

Fig.2 Annual fluctuations of different amphibian species on lakeshores of the Marii El, Russia (ASTRADAMOV & ALYSHEVA, 1979) 1: *Rana arvalis*; 2: *Rana esculenta/lessonae*; 3 *Rana ridibunda*; 4: *Bombina bombina*; 5: total number.

Source MNHN, Paris

There are some examples of deliberate introductions of Anura by man. Breeding *B. viridis* were found in 1984 in Novosibirsk City – at 650 km from the range margin of this species (ZOLOTARENKO, 1985). *R. nigromaculata* tadpoles were imported to Turkmenistan from China together with herbivorous fry fishes about 30 years ago. The frog was successfully acclimatized in the Kara-Kum Channel (ATAEV & ATAEVA, 1981).

The most marked expansions are shown by *R ridibunda*. Probably, between 1903 and 1939 this species naturally penetrated the Lake Balkhash basin (Kazakhstan) from the River Chu valley (Kyrgyzstan) (KORELOV, 1953). Between 1964 and 1970 the species extended eastwards: into the surroundings of Uch-Aral Village (Alakul Hollow, Kazakhstan) (GRACHEV, 1971). Subsequent dispersal of the species was conditioned by anthropogenic factors: in the European steppe and desert regions, by land-reclamation; in Siberia, by thermal pollution of the environment (pouring out of warm waters into the permanent reservoirs). In the 1960s, the frog was introduced with fry fishes to Issyk-Kul Lake from River Chu valley, Kyrgyzstan, and started to displace local *Rana asiatica*. In 1969, tadpoles were imported with fry fishes from Krasnodar (Southern Russia) into the city of Verkhny Tagil (Ural); since 1976, the population has expanded into neighbouring rivers (TOPORKOVA, 1977, 1978). Introductions to other Uralian cities (Ekaterinburg in 1977 and Chelyabinsk in 1981) led to new populations being established (VERSHININ, 1990). In 1970, the species was first recorded in the city of Gorno-Altaisk (Altai Mountains, Eastern Siberia), where it was introduced from the Osh Province (Kyrgyzstan) with fishes. Since that time, a stable population of *R. ridibunda* has been established, which has displaced local *B. bufo* and *R arvalis* at some sites (YAKOVLEV, 1990). At the same time *R. ridibunda* was introduced into ponds in Yakutsk City (BELIMOV & SEDALISHCHEV, 1980) Introductions in Kazakhstan (cities of Karaganda, Pavlodar and Ust-Kamenogorsk) were probably related to the release of frogs from local universities and institutes (PRUS & SMOLYANINOVA, 1989)

PERSPECTIVES

Quantitative data on declining amphibian populations in the former U.S.S.R. are not numerous. Nevertheless, existing records show declines to be widespread. Anthropogenic factors play the main role in this. Inexplicable declines, or extensive short-term declines under natural factors, have not been registered here. The increase and dispersal of several species under anthropogenic influences have taken place against a general background of impoverishment of regional amphibian assemblages "Perestroika" and the dismemberment of the U.S S.R. have led to economic and political chaos. The latter may result in both the retardation of environmental destruction due to the closing of factories, and in non-controlled destruction of nature in other sites due to violation of laws The latter may already be occurring, e.g. in an unprecedented trade in amphibians, including protected species.

In 1992, a C.I.S. Regional Group was established within the Declining Amphibian Populations Task Force of I.U C N This Group consists of amphibian biologists working in 10 Sub-Regional Groups. We look forward to consolidating our studies on amphibian declines within the territory of the former U.S.S.R.

Source MNHN, Paris

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Ionic net fluxes through the *in situ* epithelia of larval *Caudiverbera caudiverbera* (Anura, Leptodactylidae)

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The nature of the *in vivo* epithelial exogenous Cl^- and Na^+ uptake mechanisms at gilled and gill-less stages of tadpoles of the Chilean frog *Caudiverbera caudiverbera* was studied.

Ion net fluxes (Jn) in tadpoles acclimated in tap-water (controls), deionized water and in choline.Cl and Na_2SO_4 solutions were measured.

Larvae took up both Cl^- and Na^+ from diluted NaCl solutions. Tadpoles kept in deionized water showed the highest fluxes; larvae from choline.Cl solutions showed elevated $JnNa^+$ while those from Na_2SO_4 had very high $JnCl^-$. The differences found in the Jn suggest that the ion uptake mechanisms might be partially independent.

Acclimation solutions slightly affected the plasma ion concentration values with respect to controls. Concentrations of Cl^- and Na^+ of animals from deionized water showed a decreasing tendency; the level of those ions was lower in plasma of tadpoles maintained in Na_2SO_4 and choline.Cl, respectively.

It was postulated that the *in vivo* ion uptake must be active.

INTRODUCTION

The mechanisms of water and ion equilibrium in amphibian larvae are not as extensively understood as in adults (see DUELLMAN & TRUEB, 1985). Several authors have summarized our knowledge on different aspects of the osmoregulation in larvae of these vertebrates (ALVARADO, 1979; WARBURG & ROSENBERG, 1990).

We attempted to improve our comprehension of the nature of the epithelial ionic uptake mechanisms during the larval ontogenetic cycle of *Caudiverbera caudiverbera*. It is a large aquatic leptodactylid endemic to Chile (CEI, 1962; DIAZ, 1983; SALIBIÁN, 1974,

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1980), characterized by the fact that after metamorphosis juveniles remain in contact with water throughout their lives. Only few anurans are known to share this unusual type of life cycle with *C. caudiverbera*.

The sequence of morphological events during metamorphosis of this species is similar to those described in the larval development of terrestrial anurans. ZAMORANO et al. (1988) studied the pattern of urinary nitrogen excretion along their larval-juvenile transition; they found a physiological peculiarity: in spite of their permanent aquatic condition, a shift from ammoniotelism to ureotelism occurs as in the water-to-land model of metamorphosis.

Our main purpose was to study the characteristics of exogenous Cl^- and Na^+ uptake mechanisms through the intact epithelia of prometamorphic and climactic larval stages of *C. caudiverbera* after acclimations in solutions of different chemical composition.

MATERIALS AND METHODS

PREPARATION OF THE ANIMALS

Larvae were collected in natural ponds at Melipilla, Chile. The animals were held in the laboratory at 18°C in aquaria containing tap-water renewed daily and fed *ad libitum* with cooked spinach. The photoperiod was LD 12/12 It has been demonstrated that the ion transport mechanisms in amphibian larvae are affected by environmental factors such as temperature (PARSONS, 1975) and season (COX & ALVARADO, 1979). Since the experiments reported here were conducted at constant temperature and photoperiod and carried out during a short period of time, it is reasonable to conclude that our results were not irregularly affected by those factors.

Before the experiments, animals were divided into four groups and left without food for 15-20 days in one of the following solutions: (1) tap-water (control group); (2) deionized water (prepared from distilled water passed through a Kotterman 7002 resin column); (3) 3.4 $mEq.l^{-1}$ choline.Cl; (4) 3.4 $mEq.l^{-1}$ Na_2SO_4. Animals tolerated acclimation conditions without signs of perturbation. In previous studies carried out on adults of *C. caudiverbera* (MORENO et al., 1978; SALIBIÁN, 1970, 1973), we have demonstrated that animals kept in tap-water could be considered as controls since the evaluated morphological and chemical parameters were identical to those of frogs maintained in NaCl dilute solutions.

The Cl^- and Na^+ concentrations in the tap-water were periodically checked during the acclimation period; they were (means $\pm$ SEM, in $mEq.l^{-1}$) 3.2 $\pm$ 0.5 (n = 12) and 2.9 $\pm$ 0.4 (n = 12), respectively.

Animals were staged adapting the notation of GOSNER (1960). The considered stages were the following: (1) gilled (or ETKIN's prometamorphic) stages: 36-37 (toes partially or totally separated) and 38-39 (metatarsal tubercle and pigment free patches on the inner surface of toes appear); and (2) gill-less (or climactic) stages: 42-43 (incomplete regression of the tail) and 44-46 (complete resorption of the tail).

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The body weight range (in grams) of tadpoles at stages 36-37 was 18.6-24 0, and 34.2-40.4 at stages 38-39, in the gill-less larvae the ranges were 28.9-34.0 (stages 42-43) and 19.5-21.3 (stages 44-46).

MEASUREMENT OF IONIC NET FLUXES

Animals were weighed in water with a digital Sartorius 2250 balance at the beginning and at the end of the experiments; no statistically significant changes were observed between these two data sets of values. A polyethylene PE 20 cannula was inserted into the cloaca of the larvae and tightly fastened by a concentric subepithelial ligature; by this means renal and intestinal contamination of the external bath was avoided.

Each cannulated tadpole was individually placed in a container with 200 ml of its acclimation solution and maintained in that condition for about 16 h; thus physiological alterations due to the manipulation stress were avoided, as it was found in our laboratory (unpublished). Then the external solution was carefully replaced by siphoning with 150-200 ml of 1.7 $mEq.l^{-1}$ NaCl solutions. Aliquots of the external bath for subsequent analyses of Cl^- and Na^+ concentrations were taken at the beginning of the experiments and every 30-45 mn during the following 6-7 h.

The Cl^- and Na^+ concentrations in those samples were plotted against time and the net fluxes calculated from the slopes of the regression lines For this calculation a mean volume of the external bath was considered. The experiments were performed in spring; each tadpole was used in only one experiment. Animals that showed abnormally high negative net fluxes during the first hour of the experiment or with signs of skin damage were discarded.

Since our flux data corresponded to animals fasted for 2-3 weeks prior to the measurements, food must be discarded as an additional source for balancing the Cl^- and Na^+ losses ALVARADO & MOODY (1970) have shown that even in the fasting condition, the gut cannot be a physiologically relevant site for ion accumulation from the external bath because the volume drunk by the tadpoles can be considered negligible.

BLOOD SAMPLING

Blood samples were drawn from animals anesthetized in 0.3 % tricaine (Sigma) solutions through a little cut in the extreme of the ventricle. Blood was collected in glass tubes with heparin (Biochimie) at 5 $IU.ml^{-1}$ of blood. Plasma was separated by centrifugation at 4°C in a Sorvall RC-2B centrifuge at 600 g for 15-20 min, and diluted 1/100 before the analyses.

ANALYTICAL TECHNIQUES

Chloride concentration was evaluated potentiometrically (SANDERSON, 1952) with a Radiometer titration unit (pHmeter 26, TTT auto titrator and autoburette type ABU-1C) or with a Buchler digital H-2500 chloridometer. Sodium concentration was determined by emission photometry with an Eppendorf flame photometer.

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Expression of the results and statistical analyses

All data are given as means ± SEM (standard error of the mean). Net ion fluxes are expressed as $\mu Eq.h^{-1}.100\ g^{-1}$ animal weight. Plasma ion concentrations are in $mEq.l^{-1}$.

Student's t test was used to test differences between means of ion net fluxes. Results of plasma Cl^- and Na^+ concentrations of tap-water, deionized water and choline.Cl animals were tested for normality (Kolmogorov-Smirnov); then a two-way analysis of variance followed by Scheffé-Dunnett tests for comparison of means were made. Plasma ion concentrations of Na_2SO_4 larvae only were tested for significant differences between 36-37 and 44-46 stages data of animals by means of Student's t test.

Results

Net ionic fluxes in control tap-water acclimated larvae (Table I)

The magnitude of Cl and Na^+ epithelial net fluxes (Jn) were similar at stages 36-37 and 42-43; in the latter the fluxes were reduced by 50 %. At the end of metamorphosis (stages 44-46), there was a further reduction and a partial dissociation of the fluxes, with $JnCl^-$ being significantly higher than $JnNa^+$; this dissociation was not observed at the two earlier stages

Net ionic fluxes in deionized water acclimated larvae (Table II)

When compared with those of controls from tap-water, the absolute $JnCl^-$ and $JnNa^+$ values in these larvae were always very high. At early stages (36-37 and 38-39), $JnNa^+$ was higher than $JnCl^-$ while the opposite occurred at the last gill-less stages of the metamorphosis.

Net ionic fluxes in choline Cl solutions acclimated larvae (Table III)

In all studied stages the incubation of animals in Na-free solutions resulted in higher $JnNa^+$ than $JnCl^-$. The differences between net fluxes remained unchanged during stages with gills, but increased abruptly later as metamorphosis advanced. It is interesting to note that in these animals the $JnCl^-$ were comparable to those of control tap-water tadpoles of the same stage.

Net ionic fluxes in Na_2SO_4 solutions acclimated larvae (Table IV)

Results show that at stage 37 both $JnCl^-$ and $JnNa^+$ were low and similar. When animals reached stages 44-46 fluxes were dissociated, with $JnCl^-$ being seven times higher than $JnNa^+$ which, in turn, was almost identical to that found in control animals of the same age

Source MNHN, Paris

Table I. - Cl^- and Na^+ net fluxes (Jn) through the *in vivo* epithelia of larval *Caudiverbera caudiverbera* acclimated in tap-water. Fluxes were measured from 1.7 mEq l^{-1} NaCl solutions; data in $\mu Eq.h^{-1}.100\ g^{-1}$ body mass (means ± SEM); N: number of experiments.

Larval stages	N	$JnCl^-$	$JnNa^+$	$JnCl^-$-$JnNa^+$ [1]	p [2]
36 - 37	15	+17.6 ± 1.3	+18.0 ± 1.9	–0.4 ± 0.1	N.S.
42 - 43	15	+9.9 ± 0.9	+10.9 ± 0.8	–1.0 ± 0.1	N.S.
44 - 46	15	+3.6 ± 0.5	+3.0 ± 0.3	+0.6 ± 0.1	< 0.025

1. Mean differences of paired data ± SEM.
2. Statistical significance of the differences.

Table II. - Cl^- and Na^+ net fluxes (Jn) through the *in vivo* epithelia of larval *Caudiverbera caudiverbera* acclimated in deionized water. Fluxes were measured from 1.7 mEq.l^{-1} NaCl solutions; data in $\mu Eq.h^{-1}.100\ g^{-1}$ body mass (means ± SEM); N: number of experiments.

Larval stages	N	$JnCl^-$	$JnNa^+$	$JnCl^-$-$JnNa^+$ [1]	p [2]
36 - 37	15	+42.7 ± 4.2	+71.4 ± 3.2	–28.7 ± 0.9	< 0.001
38 - 39	15	+23.3 ± 2.5	+32.9 ± 2.3	–9.6 ± 0.5	< 0.025
42 - 43	17	+23.1 ± 1.9	+21.8 ± 2.1	+1.3 ± 1.0	N.S.
44 - 46	17	+57.3 ± 1.6	+29.5 ± 1.1	+27.8 ± 0.9	< 0.001

1. Mean differences of paired data ± SEM.
2. Statistical significance of the differences.

Table III. - Cl^- and Na^+ net fluxes (Jn) through the *in vivo* epithelia of larval *Caudiverbera caudiverbera* acclimated in 3.4 mEq.l^{-1} choline Cl solutions. Fluxes were measured from 1.7 mEq.l^{-1} NaCl solutions; data in $\mu Eq.h^{-1}.100\ g^{-1}$ body mass (means ± SEM); N: number of experiments.

Larval stages	N	$JnCl^-$	$JnNa^+$	$JnCl^-$-$JnNa^+$ [1]	p [2]
36 - 37	8	+20.2 ± 2.0	+28.9 ± 1.5	–8.7 ± 2.3	< 0 01
38 - 39	10	+21.7 ± 0.7	+29.4 ± 2.8	–7.7 ± 2.5	< 0.025
42 - 43	8	+2.3 ± 0.4	+14.9 ± 1.6	–12.6 ± 0.9	< 0.001
44 - 46	13	+6.1 ± 1.4	+27.0 ± 2.3	–20.9 ± 1.0	< 0.001

1. Mean differences of paired data ± SEM.
2. Statistical significance of the differences.

Source MNHN, Paris

Table IV. - Cl^- and Na^+ net fluxes (Jn) through the *in vivo* epithelia of larval *Caudiverbera caudiverbera* acclimated in 3.4 mEq.l^{-1} Na_2SO_4 solutions. Fluxes were measured from 1.7 mEq.l^{-1} NaCl solutions; data in µEq.h^{-1}.100 g^{-1} body mass (means ± SEM); N: number of experiments.

Larval stages	N	$JnCl^-$	$JnNa^+$	$JnCl^-$-$JnNa^+$ [1]	p [2]
37	7	+2.9 ± 0.2	+2.8 ± 0.3	+0.1 ± 0.1	N.S.
44 - 46	7	+19.7 ± 0.7	+2.7 ± 0.4	+17.0 ± 0.1	< 0.001

1. Mean differences of paired data ± SEM.
2. Statistical significance of the differences.

Table V. - Plasma chloride and sodium concentration of *Caudiverbera caudiverbera* larvae acclimated in different solutions. Data in mEq.l^{-1} (means ± SEM); in parenthesis, number of animals; N.M.: not measured.

Ion	Larval stages	Acclimation solutions			
		Tap-water	Deionized water	Choline.Cl (3.4 mEq l^{-1})	Na_2SO_4 (3.4 mEq.l^{-1})
Cl^-	36-37	68.5 ± 1.6 (10)	56.4 ± 3.2 (10)[2]	71.6 ± 1.7 (10)	63.0 ± 1.6 (7)
	38-39	69.3 ± 1.8 (10)	63.8 ± 2.0 (10)	75.6 ± 2.4 (10)	N.M
	42-43	71.1 ± 0.4 (13)	74.2 ± 1.7 (10)[1]	70.9 ± 2.0 (8)	N.M.
	44-46	75.4 ± 1.1 (10)[1]	69.3 ± 0.3 (11)[1]	75.4 ± 2.1 (10)	66.3 ± 2.2 (7)
Na^+	36-37	79.6 ± 3.0 (10)	72.6 ± 5.6 (10)	76.2 ± 1.6 (8)	87.3 ± 3.5 (7)
	38-39	82.2 ± 2.6 (10)	67.5 ± 1.7 (10)[2]	78.1 ± 2.4 (10)	N.M.
	42-43	96.8 ± 2.7 (13)[1]	87.3 ± 1.2 (10)[1]	86.5 ± 2.5 (8)[1]	N.M.
	44-46	106.3 ± 2.4 (10)[1]	97.9 ± 2.5 (11)[1]	95.2 ± 1.8 (10)[1]	105.6 ± 2.6 (7)[3]

1. Within each acclimation solution, significantly different from the 36-37 stage larvae ($p < 0.05$).
2. Within each larval stage range, significantly different from the tap-water larvae ($p < 0.05$)
3. Significantly different from the 36-37 stage larvae ($p < 0.001$).

Source MNHN, Paris

PLASMA Cl^- AND Na^+ CONCENTRATIONS (TABLE V)

In comparison to the Cl^- concentrations in plasma of control larvae, there was a trend towards its reduction in animals from deionized water and Cl-free solutions. The Cl^- concentration in plasma of larvae kept in choline.Cl solutions remained unaltered with respect to tap-water controls.

When compared with control tap-water acclimated tadpoles, the Na^+ plasma concentration of animals from deionized water and Na-free solutions showed a tendency to decrease; the plasma Na^+ concentration of Na_2SO_4 acclimated animals was not different from those of controls.

In all groups of animals the plasma Na^+ concentrations showed a significant increase in the gill-less stages whereas the Cl^- concentrations did exhibit a similar but less pronounced trend

DISCUSSION

Both gilled and gill-less tadpoles of *Caudiverbera caudiverbera* showed the capacity to take up Cl^- and Na^+ from dilute external NaCl solutions. This ability was found to exist independently of the age of the animals and of the acclimation conditions.

Our preparation does not allow to discriminate precisely the sites where transport occurs at each stage. However, evidence reported by several authors (see ALVARADO, 1979) indicates that during anuran metamorphosis there are gill-to-skin shifts as site for ion translocations. In tadpoles of *C. caudiverbera* indirect evidence in favor of the postulated shift along the larval-juvenile transition from the gill as the dominant organ towards the skin as the main site for ion transport was reported by GONZALEZ et al (1979) They measured the specific activity of ouabain sensitive (Na^+ + K^+)-ATPase in isolated gills and skin of larvae at different stages of animals stored in tap-water. At stages 30-35 they showed the existence of enzymatic activity only in gills; at the intermediate stages 36-39 the activity was found both in gills and skin in the proportion of 1/3, later, close to the end of metamorphosis (stages 40-43), most of the enzyme activity was detected on the skin.

Relative to the NaCl solutions used in flux measurements, plasma of *C caudiverbera* tadpoles were always markedly hyperionic with respect to Cl^- and Na^+; the ratios of their concentrations in plasma to external bathing media varied between 40 and 62.

We conclude that our data allow us to postulate that the ion accumulation processes of larval *C. caudiverbera* are active and must be attributed to epithelial mechanisms located, according to the age of the animals, in gills and/or skin.

The acclimation condition affects the magnitude of ionic net fluxes when animals are transferred to NaCl solutions. In control tap-water animals (Table I), the $JnCl^-$ and the $JnNa^+$ were practically similar; this could be interpreted as an indication of a linked uptake of Cl^- and Na^+. However, at climactic stages a clear-cut difference among the net fluxes appeared, suggesting the existence of a differentiation in their ion uptake

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mechanisms, the animals being able to take up a fraction of the exogenous Cl^- by a Na^+-independent ionic exchange system.

After keeping tadpoles in deionized water (Table II), the ion net fluxes were augmented at least three times with respect to controls; the increases were constant and not dependent on the developmental stages. Similar response to ion-free incubation of the animals was shown in intact *R. catesbeiana* tadpoles (ALVARADO & MOODY, 1970); in the same species, BROONKOOM & ALVARADO (1971) demonstrated an increase in the (Na^+ + K^+)-ATPase activity in gill homogenate of "salt depleted" animals.

Tadpoles coming from Na-free solutions (Table III) took up Na^+ at higher rate than Cl^-, independently of the considered age. Conversely, the $JnCl^-$ in animals from Cl-free solutions (Table IV) were higher than the $JnNa^+$; the ion net fluxes were among the lowest registered in the early stages In both groups the differences between fluxes were greatly enhanced in climactic stages.

It must be emphasized that the observed differences in epithelial net ion flux values between the controls and the remaining three groups cannot be attributed to changes in the plasma Cl^- and Na^+ levels (see Table V) It might be necessary to search for that correlation in the intracellular condition of the epithelial cells involved in ion transport processes rather than in that of the extracellular fluids (COX & ALVARADO, 1983; LARSEN, 1988) The relative constancy found in the Cl^- and Na^+ concentrations of the extracellular compartment may explain the excellent tolerance of these animals to incubation for 2-3 weeks in artificial environmental conditions.

Tadpoles in ion-free media are exposed to a severe limitation in their capacity to compensate for the normal ion and water losses We postulate that the particular condition of these animals may trigger an endocrine compensatory reponse, increasing the circulatory levels of hormones involved in the hydromineral balance regulation through epithelia, especially neurohypophysial peptides and aldosterone. The integrated action of these hormones upon their effectors may act by promoting an increased reabsorption of water and ions, principally Na^+, thus reducing the losses and, consequently, maintaining the plasma levels stable over the ion deprived incubation period of time. The existence of such compensatory responses was shown in adults of several anuran species (BENTLEY, 1969; CRABBE, 1963; ROJAS et al., 1987) and in embryos of *Bufo arenarum* (CASTAÑÉ et al., 1987).

Finally, the magnitude of the *in vivo* ion net fluxes of control tadpoles tend to decrease as metamorphosis goes on and become close to those measured in adults (GARCIA ROMEU et al., 1969). This fact might be the consequence of the reduction in the ion exchange areas due to resorption of the gills and tail and/or ontogenetic selective modifications in the magnitude of the unidirectional fluxes.

RESUMEN

Se estudió la naturaleza de los mecanismos epiteliales de captación *in vivo* de Cl^- y Na^+ exógenos en estadios larvales branquiados y no branquiados de larvas de la rana chilena *Caudiverbera caudiverbera*.

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Se midieron los flujos iónicos netos (Jn) en larvas aclimatadas en agua potable (controles), agua deionizada y en soluciones de Cl.colina y SO_4Na_2.

Los animales captaron Cl^- y Na^+ desde soluciones diluidas de ClNa. Los Jn más altos se observaron en larvas de agua deionizada; las de Cl.colina tuvieron los $JnNa^+$ elevados mientras que los $JnCl^-$ fueron mayores en las de SO_4Na_2. Las diferencias halladas en los Jn sugieren que los mecanismos de captación iónica podrían ser parcialmente independientes.

Las soluciones de preadaptación afectaron ligeramente las concentraciones plasmáticas de Cl^- y de Na^+ con respecto a las de los controles. Las de los animales de agua deionizada mostraron una tendencia a la reducción; los niveles de esos iones resultaron disminuídos en larvas mantenidas respectivamente en SO_4Na_2 y Cl.colina.

Se postuló que el transporte de iones *in vivo* debe ser de carácter activo.

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